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Three Ascending Spinal Pathways in the Dorsal Part of the Lateral Funiculus

By

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Abstract

LUNDBERG, A. and O. OSCARSSON. *Three ascending spinal pathways in the dorsal part of the lateral funiculus.* Acta physiol. scand. 1961. *51.* 1—16. — Three pathways with axons ascending in the dorsal part of the lateral funicle have been identified as not belonging to the dorsal spino-cerebellar tract because the axons could be activated on stimulation of the lateral funicle in L5 below the caudal level of Clarke's column but not antidromically from the anterior cerebellar lobe cortex. One of these pathways is located medial to the dorsal spino-cerebellar tract and is activated exclusively by tactile stimuli from small skin fields.

The activity in ascending pathways of the dorsal part of the lateral funicle (Flechsig's fasciculus) was analyzed by mass discharge and unit recording (LAPORTE, LUNDBERG and OSCARSSON 1956 a, b, LAPORTE and LUNDBERG 1956). In a previous report units belonging to the dorsal spino-cerebellar tract (DSCT) were identified by antidromic stimulation from the anterior cerebellum (LUNDBERG and OSCARSSON 1960). The present paper deals with the identification of three ascending pathways also with axons in the dorsal part of the lateral funiculus, but not belonging to the DSCT. Identification was achieved not only because these axons could not be activated antidromically from the cerebellum but also from the finding that they were activated by a stimulus applied to the lateral funicle below the caudal end of Clarke's column (REXED 1954). Evidence that there are other ascending pathways in Flechsig's fasciculus than the DSCT has been presented by BRODAL and REXED 1953, GRUNDFEST and CARTER 1954, WALL (1960), ECCLES, ECCLES and LUNDBERG (1960). A preliminary report of these results has been published (LUNDBERG and OSCARSSON 1959).

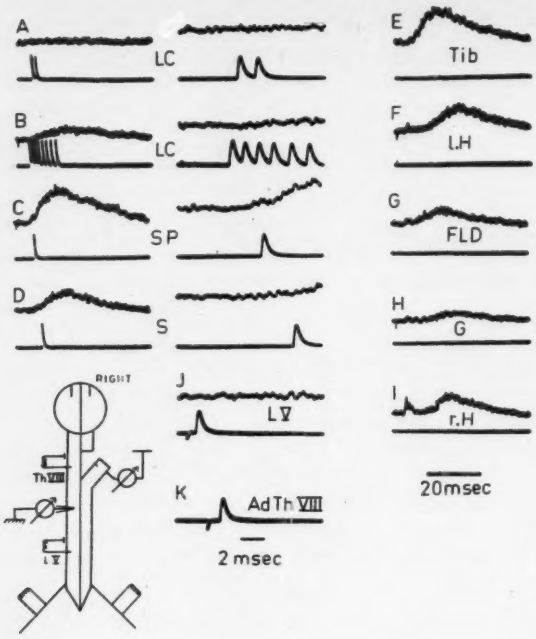

Fig. 1. Microelectrode recording (lower traces) from an axon of the tactile tract and mass discharge recording (upper traces) from the contralateral spinal half. The left and right traces in records A—D were taken simultaneously at different speeds, E—I were taken at the lower speed and J and K at the faster. Records B—I show the effect of supramaximal stimulation of the nerve indicated in each record. Stimulation in A was supraliminal. Abbreviations: L C, lateral cutaneous; S P, superficial peroneal; S, sural; Tib, tibial; l. H, hamstring; FLD, flexor digitorum longus; G gastrocnemius-soleus (all left nerves); r.H, right hamstring. Stimulation of the fibre by stimulus applied in L5 and Th8 is shown in J and K.

Distance from site of microelectrode recording to stimulating electrode in L5 4.7 cm and to stimulating electrode in Th8 7.3 cm.

Methods

The experiments were performed at the same time as the investigations reported in a previous paper on the dorsal spino-cerebellar tract (LUNDBERG and OSCARSSON 1960) and the methods were identical with those described in this paper. The experiments were made on unanaesthetized cats decerebrated by intercollicular section. Microelectrode recording was made from axons in the dorsal part of the left lateral funicle in L1. For control the mass discharge was recorded in the lower thoracic region from the contralateral right spinal half or from the dissected right ventral quadrant.

Results

1. *The tactile tract*

a) *Unit recording*

These neurones constitute an easy identifiable and homogenous group. The axons conduct at 100—60 m/sec. At electrical stimulation they are activated only from cutaneous nerves and not by muscle afferents. This is illustrated in Fig. 1 for a unit receiving its main activation from the lateral cutaneous nerve (which penetrates the lower part of biceps and innervates the lateral part of the leg below the knee). Record A shows the effect of stimulation of this nerve

at threshold strength, which does not give rise to a mass discharge in the contralateral spinal half (upper trace); at stronger stimulation in B there is a train of 8 impulses. In all of these neurones the latency of the first spike in response to a volley in the nerve supplying the main excitation was so brief that transmission must be monosynaptic. Record C and D, Fig. 1, show that there was some subsidiary excitation from the superficial peroneal (C) and the sural (D) nerve, with one spike on supramaximal stimulation of each of them. There was no effect on supramaximal stimulation of any of the ipsilateral muscle nerves F—H, or from the mixed tibial nerve (E). Stimulation of contralateral nerves did not in any case evoke excitation (I, Fig. 1). These axons could not be antidromically activated from the cerebellum but were stimulated by a shock applied to the lateral funicle in L5 (record J, Fig. 1) which is below the caudal level of Clarke's column (REXED 1954).

When tested for adequate activation these units were found to respond extremely effectively (often at frequencies above 500/sec) to light touch, all of them were in fact activated by blowing of hairs. It was not possible to observe any increased activation on pressure or pinching of the skin and thereby these units differ radically from the two subgroups of the DSCT (LUNDBERG and OSCARSSON 1960) and also from units described in section 2 of this paper of which many are activated by tactile stimuli. The receptive fields were usually quite small, with many of the units activated from the toes, excitation was provided from a skin area of about 10 mm^2; more proximally on the limb larger receptive fields were found and on the proximal part of the thigh or the lower trunk they could be as large as 20 cm^2. In this connection it should be considered that receptive fields of primary tactile afferent diminish in a distal direction (YAMAMOTO, SUGIHARA and KURU 1956, LINDBLOM 1958 cf., however, WALL, 1960) and that similar findings have been made on units in Flechsig's fasciculus (YAMAMOTO and MIYAJIMA 1959) and also on cells in the cats sensory cortex (MOUNTCASTLE 1957).

These tactile units have a resting discharge (like the neurones of all other pathways we have investigated) against which inhibition easily can be disclosed. However, there was never any inhibition on touch, pressure and pinching of the skin of the hindlimbs and the trunk. Identical findings were made by WALL (1960) on tactile cells in the dorsal horn of the lower lumbar region. In summary the organization of connections to the tactile tract is rather simple, it forwards a modality specific message which is highly spatially discriminative.

b) *Contribution to mass discharge and location of pathway in Flechsig's fasciculus*

This investigation of non-DSCT pathways in the dorsal part of the lateral funicle, was in part based on a comparison of evoked cerebellar potentials and the discharges in dissected Flechsig's fasciculus (LAPORTE *et al.* 1956 a, LUNDBERG and OSCARSSON 1960). On stimulation of skin nerves (cf. Fig. 5 B and D, LUNDBERG and OSCARSSON 1960) no correspondence was found in the

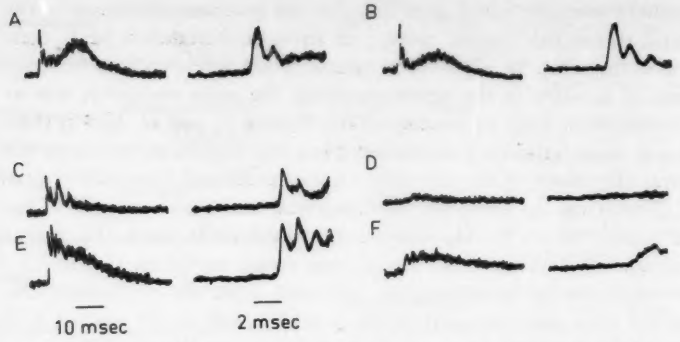

Fig. 2. Mass discharge recording at two speeds from the left dissected Flechsig's fasciculus in Th 10 in a spinal cat. The left hamstring nerve was stimulated supramaximally in A and B. The left superficial peroneal nerve was stimulated at a strength of 1.03 times threshold in C and D and at 20 times threshold in E and F. Records B, D and F were taken after section of the dorsal part of the left lateral funicle in the upper part of L5.

evoked potential from the anterior cerebellum to the well synchronized fast discharge with a steep front which characterizes the recording from the dissected Flechsig's fasciculus and is monosynaptically transmitted from the primary cutaneous afferents (LAPORTE *et al.* 1956 a).

Fig. 2 shows directly that the well synchronized early cutaneous mass discharge is due to activity in pathways not belonging to DSCT. In record C the superficial peroneal nerve was stimulated at a strength 1.03 times threshold and in E at 20 times threshold. The corresponding records D and F were obtained at the same relative strengths after ipsilateral sectioning of the dorsal part of the lateral funicle in L5. The fast discharge and the successive waves of well synchronized volleys has disappeared. With respect to the discharge evoked from the hamstring nerve (A and B) there is no significant change of the early spike-like discharge due to activity in DSCT fibres activated by group I afferents but the late mass discharge is reduced.

In the experiments with microelectrode recording from the axons of the tactile pathway it was observed that they were located very medially in Flechsig's fasciculus and the DSCT axons more laterally. It is possible to demonstrate a separate location of the DSCT axons and the axons responsible for the fast cutaneous discharge, also with mass discharge recording. In the experiment of Fig. 3 these discharges were recorded with steel-electrodes, insulated except for the tip, which was about 10 μ in diameter. Two recording positions were used as shown in the diagram of Fig. 3. The upper records were obtained from the lateral position 1.3 mm from the dorsal column, and the lower records from the medial position, 0.2 mm from the dorsal column (the dorsal column was transected at a more caudal level). The cutaneous

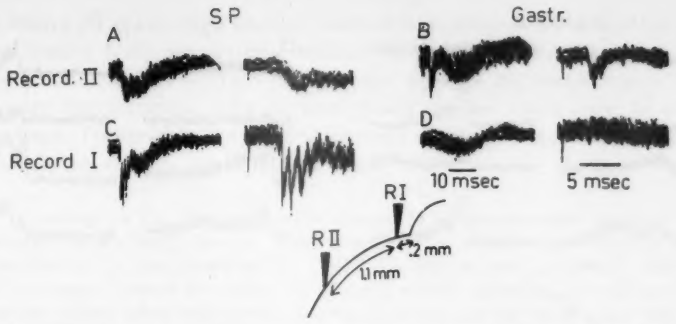

Fig. 3. Recording (at two speeds) from the surface layer of Flechsig's fasciculus in Th 11 with steel-electrodes, electrolytically pointed to have a tip diameter of about 10 μ. Positivity is signalled downwards. Record A and B were obtained from the lateral and C and D from the medial recording position (see drawing). The dorsal column was transected at a more caudal Supramaximal ipsilateral stimulation of the supraficial peroneal (SP) nerve in A and C and of the gastrocnemius-soleus (Gastr.) nerve in B and D.

superficial peroneal nerve was stimulated in A and C and it is clear that the discharge with well synchronized successive volleys is found only in the more medial recording position (record C). On the other hand on stimulation of the nerve to gastrocnemius-soleus the group I evoked DSCT discharge is hardly visible when recording medially (record D) but is large in the lateral recording position (record B). Slow mass discharges can be observed in all the records and are similar to those recorded from the dissected fasciculus.

It is also possible to demonstrate the separate location of the DSCT and the tactile tract by recording from dissected fasciculi. The records in Fig. 4 are from an experiment in which one medial and one lateral filament were dissected from Flechsig's fasciculus approximately as shown in the diagram. In each record of Fig. 4 the upper traces were obtained from the medial and the lower traces from the lateral fasciculus. On stimulation of the hamstring nerve (at 2 times threshold which was slightly supramaximal for group I in A and at 30 times threshold in C), a large group I evoked DSCT discharge could be recorded in the lateral fasciculus but not in the medial fasciculus. When stimulating the cutaneous superficial peroneal nerve (in B at the supraliminal strength of 1.05 times threshold and in D at 20 times threshold), the early well synchronized cutaneous discharge is large in the medial fasciculus (right upper trace in B and D); there is a corresponding deflection also in the lateral fasciculus (right lower traces in B and D) but the size is only 20 % of that found in the medial recording. Records E, F and G were taken after a lesion in the dorsal part of the lateral funicle in L5. As in Fig. 2 the group I evoked DSCT discharge (F) is unchanged but the cutaneous discharge in the medial fasciculus (right upper traces in E and G as well as the corresponding

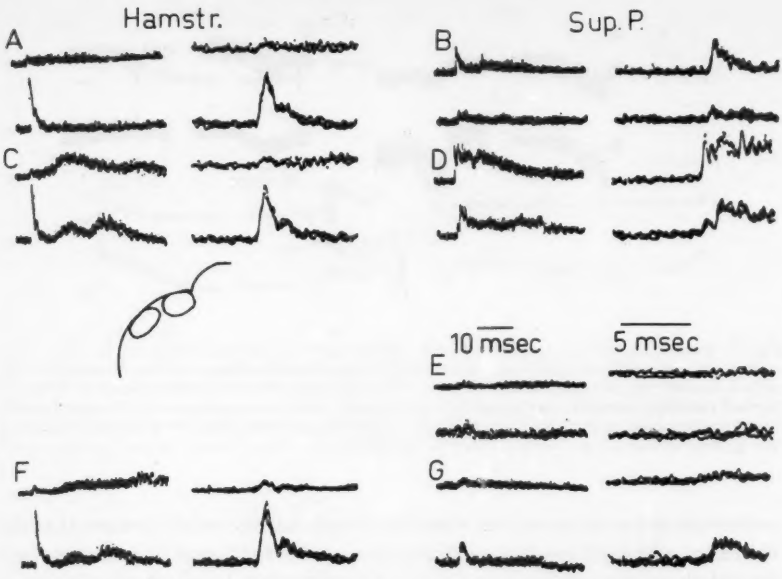

Fig. 4. Simultaneous recording in the lower thoracic region from two dissected filaments of Flechsig's fasciculus in a spinal cat. One medial and one lateral fasciculus was dissected as shown in the drawing and in each record the upper trace is from the medial and the lower from the lateral fasciculus. The ipsilateral hamstring (Hamstr.) nerve was stimulated in A, C and F, in A at a strength of 2 times threshold and in C and F at 30 times threshold. Stimulation of the ipsilateral superficial peroneal (Sup. P.) nerve in B, D, E and G, in B and E at a strength of 1.05 and in D and G at 20 times threshold. Records E, F and G were taken after section of the dorsal part of the lateral funicle in L5.

small early cutaneous discharge in the lateral fasciculus have disappeared. It should be observed that the main cutaneous discharge in the lateral fasciculus in D and the corresponding remaining discharge in G after the lesion have their onset 0.8 msec later than the early cutaneous discharge. This discharge in the lateral fasciculus is probably mainly due to activity in DSCT neurones monosynaptically activated by cutaneous afferents (LUNDBERG and OSCARSSON 1960) but a contribution from the pathway described in the next section cannot be excluded (cf. below). A longer latency would be expected in DSCT units because of the relatively slow conduction in the dorsal column in which the cutaneous afferents ascend to reach Clarke's column. The difference of 0.8 msec in the experiment of Fig. 4 was exceptionally long, the usual difference was 0.3 msec.

The pathway described in the next section is also monosynaptically activated by cutaneous afferents and should contribute to the non-DSCT cuta-

neous discharge in Flechsig's fascicle. Most of these axons were located medially but we cannot from our material exclude the possibility that they are more widely distributed in Flechsig's fascicle than those of the tactile tract. These units were not common, only 12 were found against more than 50 of the tactile tract. Presumably the early cutaneous discharge recorded medially is due largely to activity in the tactile tract.

The recordings in Fig. 3 are of special interest in connection with the early work on pathways in Flechsig's fasciculus and may serve to explain some findings which have puzzled us. In their work on the DSCT, GRUNDFEST and CAMPBELL (1942) recorded discharges evoked by volleys in group I muscle afferents, but did not then observe any effects when stimulating cutaneous nerves. On the other hand when recording from the dissected Flechsig's fasciculus the characteristic cutaneous discharge is always found (LAPORTE *et al.*, 1956 a). Presumably GRUNDFEST and CAMPBELL (1942) recorded laterally in the true location of the DSCT where the cutaneous discharge is not so conspicuous and may have escaped notice. LLOYD and MAC INTYRE (1950) based their analysis of the DSCT on GRUNDFEST's and CAMPBELL's finding that no discharge was evoked from cutaneous afferents. They stimulated the dorsal roots and assumed that discharge recorded in the ascending pathway was evoked only by muscle afferents. The discharge recorded by them was different from that found by GRUNDFEST and CAMPBELL and from discharge now known to be due to activity in the DSCT (records A and B, Fig. 3), but it is very similar to the discharge in record C, Fig. 3. Their recording position probably was medial and the discharge found by them probably due to activity in the tactile tract. It should also be mentioned that GRUNDFEST and CARTER (1954) have found a cutaneous discharge in the lateral funicle which they ascribe to activity in a spino-olivary pathway.

c) *Is transmission to the tactile tract supraspinally controlled?*

For the understanding of the function of any pathway it is important to learn about the supraspinal controlling systems which may influence transmission. We have not made an exhaustive study of the control of the tactile tract but have investigated if any of the two supraspinal control systems analysed by HOLMQVIST, LUNDBERG and OSCARSSON (1960 a, b) has an effect. One of these systems takes origin in the anterior cerebellum, relays in the brain stem and activates monosynaptically the cells of a ventral pathway (HOLMQVIST, LUNDBERG and OSCARSSON 1960 b). This system does not connect with the tactile pathway as evidenced by the finding that the resting discharge in these units is not influenced by repetitive stimulation of the anterior cerebellum. Neither was there any effect on transmission to this pathway, tested with the single volley technique and with adequate activation. Another supraspinal control system inhibits effectively the interneurones of the flexion reflex pathway and the interneurones mediating effects to a number of ascending pathways influenced by the flexion reflex afferents (HOLMQVIST and LUNDBERG 1959, HOLMQVIST, LUNDBERG and OSCARSSON 1960 a). This control system can be activated by stimulation of the dorsal half of either lateral funicle. This was tried with 15 of the neurones of the tactile tract both with

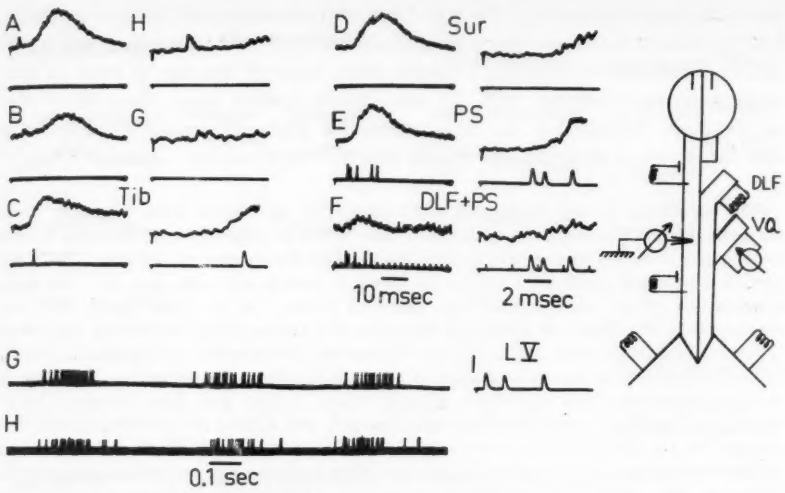

Fig. 5. As in Fig. 1 microelectrode recording from axon of the tactile tract with simultaneous recording of mass discharge in the contralateral dissected ventral quadrant. Stimulation of various hindlimb nerves in A—F. Abbreviations different from those in Fig. 1 are: H, left hamstring, Sur., sural; PS, superficial peroneal. In F the superficial peroneal nerve was stimulated during repetitive stimulation of the dissected dorsal half of the right lateral funicle (DLF). Observe that there is no effect on the unit discharge while the mass discharge in the contralateral spinal half is strongly depressed. The receptive skin field was on the dorsum of the two lateral toes. In G and H light touch was applied three times, in G before and in H during repetitive stimulation of the contralateral DLF. Record I shows activation of axon by a stimulus applied to the lateral funicle in L5.

the electrically and adequately evoked discharge as is illustrated in Fig. 5. Record E shows that this neurone was activated from the superficial peroneal nerve and there was also one spike on stimulation of the tibial nerve. In record F the superficial peroneal nerve was stimulated during tetanic stimulation of the dissected dorsal half of the right lateral funicle (DLF), but the discharge is unchanged. The upper trace in F shows that transmission of the mass discharge to the dissected right ventral quadrant was very much depressed and this shows that the descending control operated (cf. HOLMQVIST *et al.* 1960 a). The receptive skin field for this unit was on the dorsal side of the lateral toes and in both G and H light touch was applied to this toe 3 times, in G before and in H during tetanic stimulation of the right DLF. There was no significant change in the effectiveness of the adequate stimulation. However in 7 of the 15 investigated neurones stimulation of the contralateral DLF had a slight inhibitory effect. This effect was probably due to removal of late polysynaptic components. Our interpretation of the finding is that transmission to the tactile tract escapes this control system.

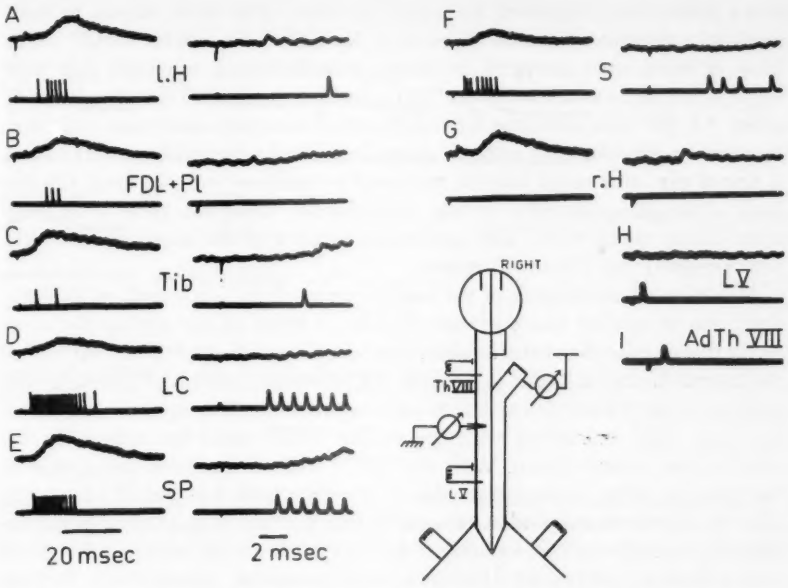

Fig. 6. As in Fig. 1 but recording from an axon activated by cutaneous and by high threshold muscle afferents. Records A—G show the effect of supramaximal stimulation of the nerve indicated in each record. Additional abbreviation from Fig. 1 are: FDL + Pl, Flexor digitorum longus and plantaris. In record H and I the axon was activated by a stimulus applied as indicated. Distance from site of microelectrode recording to stimulating electrode in L5 4.7 cm and to stimulating electrode in Th8 7.3 cm.

2. *A pathway activated by the flexion reflex afferents*

This is one of the many pathways influenced by the flexion reflex afferents (cf. HOLMQVIST *et al.* 1960 a). It seems to be a twin pathway to one of the DSCT subgroups and is likewise excited by cutaneous and by high threshold muscle afferents from a very wide ipsilateral receptive field as is illustrated in Fig. 6. Activation of the fibre by a weak stimulus applied to the lateral funicle in L5 is shown in record H. Record A and B show that there was excitation from muscle nerves but this unit received its main activation from cutaneous nerves. Single volleys from the lateral cutaneous (D), superficial peroneal (E), and sural (F) nerves evoked high frequency trains of impulses as contrasted to the more restricted fields of supply to the tactile units. The short latencies of the first spike in D, E and F demonstrate monosynaptic action, and all except one of these units was monosynaptically excited from at least one of these cutaneous nerves. However, additional polysynaptic cuta-

neous effects were indicated by longer latencies. Also with respect to their mode of adequate activation these units resembled the similar DSCT units. Most of them were activated by tactile stimuli from a restricted skin field which could be as small as few cm^2 but sometimes comprised very much larger areas. All the units activated by tactile stimuli received additional excitation on pressure and pinching and the receptive fields for these effects were larger. A few of the units could only be activated by pressure and pinching. On the basis of the adequate effect it was very easy to distinguish these units from those of the tactile tract. The conduction velocity of the axons of this pathway ranged from 100 to 40 m/sec.

The effect of stimulation of the anterior cerebellum was tested on all these units and in no case was there any significant effect on the resting discharge or on transmission from the hindlimb nerves. Stimulation of the dorsal half of the lateral funicle in order to activate the inhibitory control of interneurones mediating the flexor reflex actions to various pathways (HOLMQVIST *et al.* 1960 a), has been tried extensively with the similar DSCT units but only with few units of the present group. With the DSCT units it has been found that in the majority of the units transmission of the effect from skin (tested adequately and by electrical stimulation of various skin nerves) largely escapes the inhibitory control whereas excitation from high threshold muscle afferents is very effectively suppressed (LUNDBERG and OSCARSSON, unpublished). Similar findings were made with the two non-DSCT units tested in this respect, in one the skin effect was unchanged, in the other unit there was some reduction of the discharge evoked from skin as well. In addition it has been found with intracellular recording from two cells of this pathway that the EPSP evoked by volleys in high threshold muscle afferents was completely removed by repetitive stimulation of the dorsal half of the lateral funicle on the other side (LUNDBERG and VOORHOEVE, unpublished). The two pathways are probably similar also in this respect. When the supraspinal control operates they are apparently converted to exteroceptive pathways. The reason why the effect from skin to a large extent escapes the control is probably that it depends mainly on monosynaptic connections. However, we have no knowledge of the relative importance of mono- and polysynaptic connections to these pathways. Alternatively it would have to be assumed that the supraspinal control selects at an interneuronal level.

It is not possible to ascertain the contribution of this pathway to the mass discharge in Flechsig's fasciculus, as has already been discussed for the cutaneous effects in section 1. The activity evoked on stimulation of high threshold muscle afferents should contribute to the late component of the mass discharge. In Fig. 2 (A and B, left traces) it can be observed that this component is reduced by 50 per cent after the lesion in the lateral funicle in L5. However, it cannot be assumed that this reduction is due entirely to the interruption of the present pathway because cells of Clarke's column could receive synaptic action from collaterals of propriospinal axons ascending in the dorsal part of the lateral funicle (SZENTÁGOTHAI and ALBERT 1955, SZENTÁGOTHAI, personal

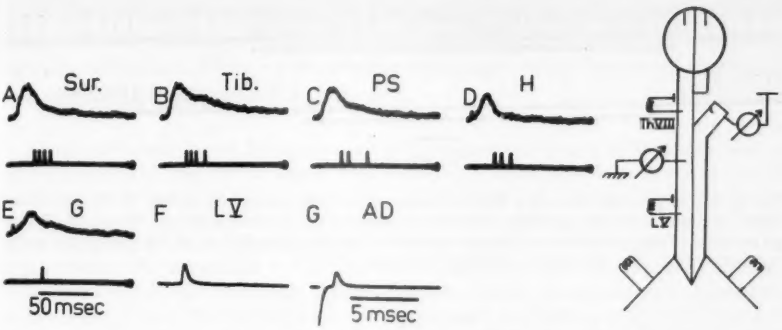

Fig. 7. As in Fig. 6 but recording from an axon activated by cutaneous and high threshold muscle afferents after a long latency. Distance from site of microelectrode recording to stimulating electrode in L5 5.5 cm and to stimulating electrode in Th8 6.8 cm.

communication). On the other hand it is not even possible to conclude that the remaining mass discharge in B, Fig. 2, is due entirely to activity in DSCT because non-DSCT axons (of this and the next section) activated by hindlimb afferents may arise rostral to L5.

3. *Neurones activated by the flexion reflex afferents after a long central delay*

In our previous work these units have not been recognized as a separate group but have been classified with the other units activated by the flexion reflex afferents. Their axons conduct at 110—70 m/sec. They are distinguished not only by the longer latencies of the effects evoked from hindlimb nerves but also by being strongly influenced from the anterior cerebellum. Usually they were activated from all ipsilateral hindlimb nerves tested and after a latency of 10—30 msec (Fig. 7 A—E). Often there was only 2—4 spikes but in more excitable preparations trains of 20—30 spikes were observed. The latency of the discharge is so long that it could be descending, but since the same response also was found in spinal animals it is concluded that we are dealing with an ascending pathway. Many of these units received excitation also from contralateral nerves although in the dorsally located ones always less than from the ipsilateral side. This is an exception to the rule that all ascending pathways with axons in the dorsal part of the lateral funicle have an ipsilateral receptive field (Oscarsson 1958, Holmqvist *et al.* 1960 a). Axons of this type are found also more ventrally in the cord and in these units the contralateral effects were more pronounced. Like the other two groups described in this paper these units were identified as not belonging to the DSCT because the axons could be activated by a stimulus applied to the lateral funicle in L5.

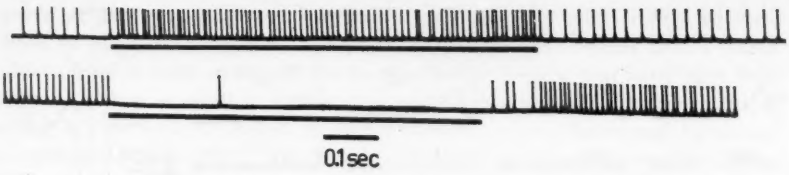

Fig. 8. Microelectrode recording from an axon of the type illustrated in Fig. 7. Upper record shows excitation during repetitive stimulation (black line) of the ipsilateral vermis of the anterior cerebellum. Lower record shows inhibition during stimulation of the ipsilateral intermediate cortex. The frequency of stimulation was 200/sec.

The effect from the anterior cerebellum was one of the criteria required to classify these units, but there was in fact cerebellar effects on all the units responding to stimulation of peripheral nerves as shown in Fig. 7. The effects were excitatory and inhibitory and in some cases it was possible to find cerebellar areas giving only one of these effects. For example, in Fig. 8 excitation (upper record) was provided from the left hemivermis and the inhibitory effect from the left intermediate cortex. It was however not possible to denote any area as preponderantly excitatory or inhibitory for these units, since there was a great variability in different units. The most common response was in fact one of mixed excitation and inhibition from the whole width of the anterior cerebellum. At the onset of repetitive stimulation there was an excitatory burst followed by inhibition and on cessation of stimulation a longlasting excitatory rebound occurred. Strong cerebellar effects have also been found on a ventral pathway, but in this case it was possible to denote separate excitatory and inhibitory longitudinal zones on the anterior cerebellum (Holmqvist *et al.* 1960 b). The possibility should therefore be considered that the effects described here are exerted by another supraspinal control system from the anterior cerebellum. Further experiments are obviously needed to investigate this problem. The cerebellar effects, described, may be of interest to account for some findings by Hagbarth and Fex (1959). They recorded from axons in Flechsig's fasciculus and observed strong effects in many ascending neurones on stimulation of the anterior cerebellum. We have not observed any marked effects on cerebellar stimulation on any of the 5 DSCT subgroups (Lundberg and Oscarsson, unpublished) or on the pathways described in the first 2 sections of this paper. It is possible that the units of Hagbarth and Fex (1959) belonged to the pathway described in this section. Alternatively they could belong to the ventral pathway described by Oscarsson (1958); this pathway is also strongly influenced from the anterior cerebellum (Holmqvist *et al.* 1960). In the lateral part of Flechsig's fasciculus axons of this pathway are sometimes found at a depth of less than 1 mm below the surface.

At descending stimulation of the contralateral dorsal half of lateral funicle there was very effective suppression of transmission to these units. This could occur without any effect on the resting discharge, which however, sometimes was inhibited but more often facilitated.

These units were found frequently and predominantly laterally in Flechsig's fascicle intermingled with the DSCT axons. Presumably they must contribute to the late mass discharge in Flechsig's fascicle. Sometimes this discharge did consist of two components as in record C, Fig. 4. The late component in the left record, with an onset 17 msec after the group I evoked discharge, could be due to activity in the units described in this section. This suggestion is not necessarily contradicted by the fact that the late component of the mass discharge is not abolished (record F, Fig. 4) after transection of the dorsal part of the lateral funicle in L5. Although not being part of DSCT some of these axons may arise rostral to L5. An additional possibility is that these axons have a more ventral location in the lower lumbar region and that some were spared by the lesion. On stimulation of the lateral funicle in L5 it was noted that many of these axons had a higher threshold than those of the other two pathways of this paper.

Discussion

Of the three non-DSCT pathways described the tactile tract deserves the greatest interest at present. These neurones are activated exclusively by light touch, and there was no additional activation on pressure and pinching as has been found with two subgroups of DSCT neurones (LUNDBERG and OSCARSSON 1960) and with the pathway described in section 2 of this paper. The very small receptive fields of the tactile tract cells makes this pathway highly spatially discriminative. The axons of the tactile tract are located in the most dorsomedial part of the lateral funicle in almost complete anatomical separation from the more laterally located dorsal spino-cerebellar tract. This may be due to the difference in segmental levels from which these pathways arise. It is known that within the dorsal spino-cerebellar tract fibres arising from higher levels of the cord are accumulated more ventrally in the tract (YOSS 1952, VACHANANDA 1959). The separate location of the DSCT and the tactile tract offer technical possibilities to differentiate between the functional significance of these pathways which may be important since a considerable number of dorsal spino-cerebellar tract neurones can be activated by tactile stimuli (LUNDBERG and OSCARSSON 1960).

Presumably the cells of origin of the tactile tract are among those intracellularly recorded from by ECCLES *et al.* (1960). In their investigation cutaneous tract cells with axons ascending in the ipsilateral dorsolateral funicle were found in the dorsal horn of the lower lumbar segment at a depth of 1.6—2.0 mm from the cord dorsum, which would be in the 4th and 5th layer of REXED (1952, 1954). All these cells received monosynaptic excitation from low threshold cutaneous afferents but some were excited by high threshold muscle afferents as well and are probably the cells of origin of the second

pathway in this paper. Others were activated exclusively by cutaneous afferents and are probably the cells of origin of the tactile tract. In some respect these cells resemble motoneurones, they have a low threshold zone in the initial segment and they also give large afterhyperpolarizations. It is not known why they unlike motoneurones respond to a single afferent volley with a high frequency train of impulses and it would be of particular interest to learn to which extent excitation is provided by polysynaptic paths. An extensive study of adequate excitation of secondary cutaneous cells has been made by WALL (1960). Some of these cells were found to send their axons up the dorsolateral funicle but since they also responded to heavy pressure of the skin they would rather correspond to the cells of our second pathway.

As to the possible functional significance of the tactile tract the work by MORIN (1955) and by CATALANO and LAMARCHE (1957) on central pathways of tactile impulses is of the greatest interest. They found that early evoked potentials of the somatic sensory areas of the cat remained after sectioning of the dorsal column but disappeared after an additional lesion in the dorsal part of the lateral funiculus. Evidence was given that the effect was relayed to the medial lemniscus in the lateral cervical nucleus of REXED and BRODAL (1951). MORIN (1955) assumed that the spinal pathway was the dorsal spino-cerebellar tract and that collaterals were given off to the lateral cervical nucleus. It now seems more likely that the ascending spinal path is the tactile tract described in this paper. Preliminary experiments have been made to find the termination of the tactile tract axons by investigating from which region they can be stimulated antidromically (LUNDBERG and NORRSELL, unpublished). They were found to terminate in the lower part of the first cervical segment, as would be required for the spinal tract of the cutaneous pathway of MORIN (1955) and CATALANO and LAMARCHE (1957).

As regards the two other non DSCT pathways it is enough at present that they have been recognized. Their functional significance should be considered in relation to their termination and the possibility that they may be propriospinal should be kept in mind.

Summary

Pathways ascending in the dorsal part of the lateral funicle have been investigated by recording mass discharges and by microelectrode recording from their axons.

Pathways have been recognized as not belonging to the dorsal spino-cerebellar tract because their axons could be stimulated in the lateral funicle in L5 which is below the caudal end of Clarke's column. These axons could not be antidromically activated from the cerebellar cortex.

Three pathways with large axons have been distinguished on the basis of sensory input and supraspinal control:

1) A pathway monosynaptically excited by the lowest threshold cutaneous afferents. These neurones respond adequately to light touch from very restricted receptive fields but do not receive additional activation on pressure and pinching of the skin. The axons of this pathway are located in the mediodorsal part of the lateral funiculus in almost complete anatomical separation from the more lateral dorsal spino-cerebellar tract. There was little or no evidence of a supraspinal control of this pathway.

2) A pathway activated by ipsilateral cutaneous and high threshold muscle afferents. These neurones resemble closely one of the subdivisions of the dorsal spino-cerebellar tract. Most of them are activated by tactile stimuli from receptive fields which for some neurones are relatively restricted but for others large. Additional activation is provided on pressure and pinching of the skin. Activation of a descending inhibitory pathway (HOLMQVIST *et al.*, 1960 a) suppresses the effects from muscle afferents converting this tract to an exteroceptive pathway.

3) A pathway excited by cutaneous and by high threshold muscle afferents after a long central delay. Strong excitatory and inhibitory effects are evoked on stimulation of the anterior cerebellum.

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From the Neurophysiological Laboratory at the Anatomical Institute, University of Oslo, Norway

Activation of the Dentate Area by Septal Stimulation

By

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Abstract

ANDERSEN, P., H. BRULAND, and B. R. KAADA, *Activation of the dentate area by septal stimulation.* Acta physiol. scand. 1961. *51.* 17—28. — In rabbits under urethane-chloralose anesthesia potentials were recorded bilaterally from the dentate area in response to stimulation of the large-celled posterior part of the medial septal nucleus. The septo-dentate response consisted of an initial negative spike followed by a slower positive wave. On the basis of recordings from the various cell layers, excitability properties, resistance to anoxia and repetitive stimulation, the initial spike is interpreted as discharges of the dentate granule cells, monosynaptically excited. The following positive wave may partly represent the subsequent spread of excitation along the dendrites of the granule cells, and partly the activation of CA4 neurons. — Stimulation of the contralateral field CA3 of the hippocampus evoked within the dentate area a potential similar in form, but with a greater amplitude and shorter latency than the septo-dentate response. This crossed CA3-dentate potential is similarly most likely due to activation of the same granule cells. The crossed projection appears to be more efficient and consists of thicker fibres than the septo-dentate path.

In the attempts to elucidate the functional role of the hippocampal region[1] increased knowledge of the sources of the afferent impulses to this region is needed. In a study of the projections from the septum it was found that single

[1] The term hippocampal region is used in the same sense as by BLACKSTAD (1956): It includes the hippocampus, area dentata, subiculum, presubiculum, parasubiculum, area entorhinalis, and area retrosplenialis *e.* The area dentata includes the fascia dentata (the molecular and granular layers) and hilus fasciae dentatae. The latter corresponds to CA4 of LORENTE DE NÓ (1934).

shock stimulation of this area in rabbits produced within the hippocampal region two distinct types of potentials, one recorded from the dentate area, and a second from the ventricular surface of field CA1, whereas no distinct potentials were obtained from the subicular and presubicular areas.

The anatomical reports on projections from the septal areas to the fornix and hippocampal region contain several controversial points which need further experimental clarification. These concern the existence of true septo-hippocampal and septo-dentate fibres as well as their exact origin, course and termination (for references see DAITZ and POWELL 1954, MCLARDY 1955 a, b, CRAGG and HAMLYN 1957, VOTAW 1960).

The present investigation represents an attempt to clarify some of these controversial issues and to study the mechanism of action of the presumable septo-fugal impulses on the dentate neurons. In a subsequent article observations concerning the septo-hippocampal projection will be presented (ANDERSEN, KAADA and BRULAND 1960).

In an independent investigation, EULER and GREEN (1960 a, b) have studied the same projections. The present study corroborates some of their findings and gives additional information concerning the origin and distribution of the septo-dentate connections.

Material and Methods

Sixteen adult rabbits anesthetized with urethane-chloralose[1] (750 and 40 mg/kg respectively) intraperitoneally, were used. The hippocampus and septum were exposed by suction of the overlying grey and white matter and covered by warm mineral oil.

Stimulation was performed with bipolar stainless steel electrodes using square wave pulses of 0.1 msec duration, a frequency of 0.3/sec, and intensities varying between threshold and five times threshold values. The recording electrodes consisted of a stainless steel wire (20—30 μ) or micropipettes (about 5μ) filled with a 3 M KCl solution. The records were obtained monopolarly, using a conventional push-pull amplifier.

Anoxia was produced in animals immobilized by intravenous decamethonium bromide (Decacurin® AFI) in doses of 0.25 mg/kg by replacement of the oxygen by nitrogen through the respirator.

The electrode tracks were identified using a modified *Nauta* method (*Andersen* 1956). The histological picture was compared with the potentials obtained from the various depths. The same staining technique was used in experiments with fibre sections to determine the extent of the lesions.

Results

Pattern of response. — Following stimulation of the septal region a typical potential was recorded bilaterally from the dentate area. This consisted of an initial sharp negative spike, followed by a slower positive wave of greater ampli-

[1] The chloralose was kindly supplied by E. Merck, Darmstadt.

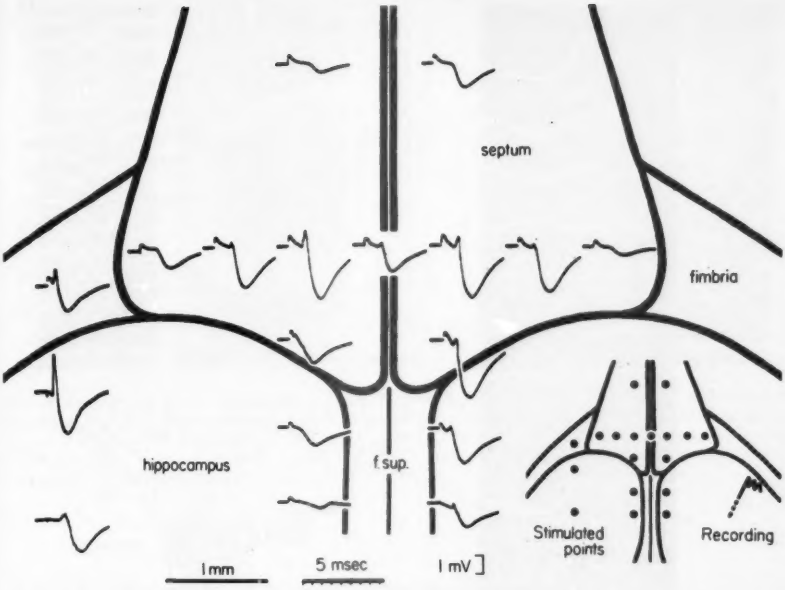

Fig. 1. *Origin of the septo-dentate projection.* Stimulation of the dorsal surface of the septum, fimbria, and hippocampus and recording from the dentate area. The potentials obtained transferred to the corresponding stimulated points as indicated by the key at the lower right.
In all figures the potentials are recorded monopolarly with negativity upwards.

tude (Fig. 1). Sometimes a late negative spike was superimposed on the positive wave. However, this spike was shown to represent the discharge of CA1 cells and will therefore not be dealt with further. The latency of the initial spike measured 2—5 msec and the duration 2—4 msec. The duration of the positive wave ranged from 10 to 25 msec and its amplitude amounted up to 7 mV. This slow wave was sometimes followed by a negative wave of lower amplitude and of a duration of 50—100 msec.

The threshold of the initial spike and the positive wave was about the same, whereas the negative wave appeared on higher stimulus strengths. On increasing intensities the positive wave showed a considerably greater increase of the amplitude than did the spike.

When a small glass capillary electrode was used for recording, the initial negative spike was at certain depths replaced by multiple small spikes (Fig. 3 D).

Occasionally the potential obtained at certain depths of the dentate area consisted of a pure negative wave of 7—15 msec duration and with a slightly longer latency than the initial negative spike (Fig. 4).

Delimitation of low-threshold structures in the septal region. — Because of the histolog-

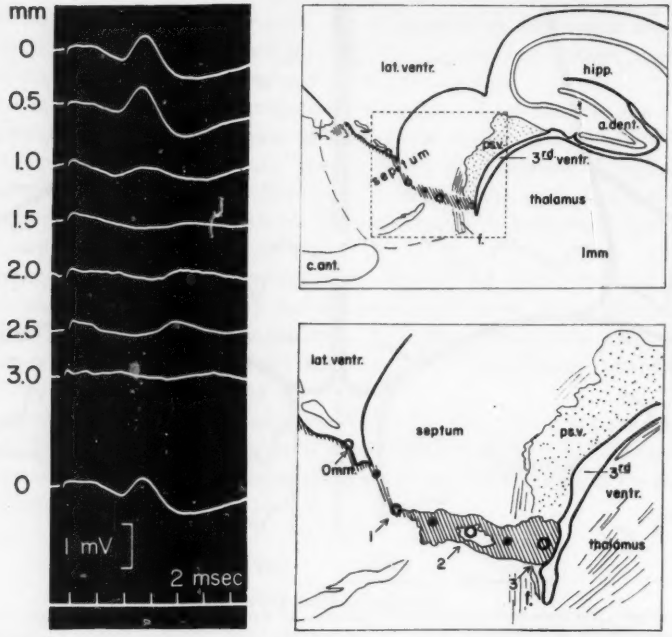

Fig. 2. *Origin of the septo-dentate projection.* Left, stimulation at indicated depths (in mm) within the septal region and recording from a fixed point in the ipsilateral dentate area. Right, drawings from a sagittal section 0.5 mm lateral to the midline demonstrating the track of the stimulating electrode in relation to adjacent structures.

ical complexity of the region stimulated it was first necessary to determine which elements were responsible for the potentials evoked in the dentate area.

By weak stimulation of the dorsal *surface* of the septum and the adjacent portions of the hippocampus and fimbria the typical response was recorded bilaterally in the dentate area when the electrode was placed on the postero-medial part of the septum (Fig. 1). Stimulation of the antero-lateral portion of the septum produced only smaller potentials. Stimulation in the midline was less effective than was stimulation 0.5 mm laterally. Inconsistent and small dentate responses were evoked from the ipsilateral fimbria and field CA3 of the hippocampus. On the other hand, excitation of the contralateral field CA3 produced a dentate potential which was similar in form but of considerably shorter latency and higher amplitude than that elicited by septal stimulation. (cf. below).

Fig. 2 shows the results obtained by stimulation at various *depths* within the septal region and recording from the dentate area. Maximum responses were obtained when the tip of the penetrating stimulus electrode was situated within

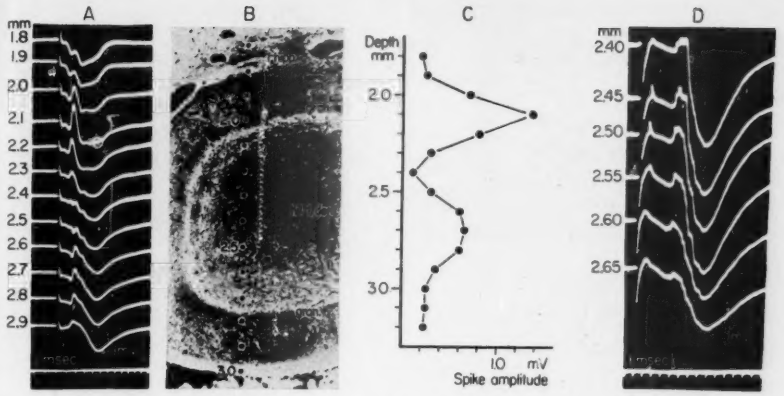

Fig. 3. *Distribution of the septo-dentate response.* Medial septal stimulation, recording at indicated depths within the ipsilateral dentate area. *A* shows two maxima of the initial spike, corresponding to the two blades of the granular cell layer in *B*. *C*, variation in amplitude of the spike at the various depths. *D* shows that the initial spike may be composed of a number of smaller spikes, and that these are recorded from a narrow zone only.

the dorsal 1 mm of the septal nuclei, whereas the response disappeared when the tip was inserted into the fornix (f.) or the ventral psalterium (ps. v.)

In conclusion, these results suggest that the septal structure yielding rise to the dentate potentials is the postero-dorsal part of the medial septal region, corresponding to the location of the magnocellular medial septal nuclei (YOUNG 1936) on both sides, and not the fornix or ventral psalterium.

Course of the septo-dentate projection. — The septo-dentate response was left unaltered by section of the fornix superior, the fimbria and the alveus between the stimulating and recording electrodes. Therefore, the fibres mediating the response is apparently situated deep to the ventricular surface of the hippocampal region, and is located between the most medial and the most lateral parts of the fornix system. Destruction of the septal nucleus on one side did not significantly affect the septo-dentate potentials recorded on either side, indicating that the projection is bilateral.

Distribution of the septo-dentate impulses. — Consistent modifications of the septo-dentate response were encountered as the recording electrode penetrated the various layers of the dentate area from the dorsal side (Fig. 3). First, the amplitude of the initial spike showed two maxima at two different recording depths; the distance between these measured from 0.6 to 0.8 mm along the usual electrode track. These maxima appear to correspond to the upper and lower blades of the granule cell layer (Fig. 3 B). When the initial single spike was replaced by a series of smaller spikes (Fig. 3 D), the amplitude of each individual

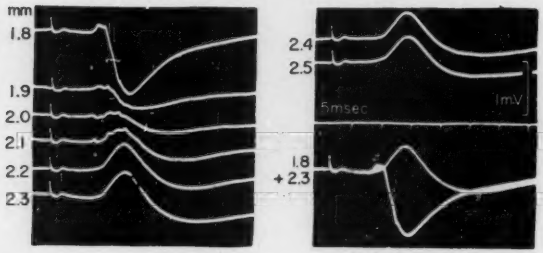

Fig. 4. *Relation between the initial spike and the following wave of the septo-dentate response.* Records obtained at indicated depths within the dentate area. In the last record the potentials obtained at 1.8 and 2.3 mm are superimposed.

spike showed the same change at the various depths, their number being constant. Second, the polarity of the initial spike changed as the electrode penetrated the dentate area. At a depth presumably corresponding to the upper blade of the granule cell layer the spike was almost purely negative (Fig. 3 A-B; 2.1 mm below the ventricular surface). Within the hilus of the dentate area the spike was almost absent (2.4 mm). At a still deeper level (2.7 mm), probably corresponding to the lower blade of the granular layer, the spike was again almost purely negative. From these observations it may be concluded that the initial negative spike of the septo-dentate response probably represents the discharge of the granule cells of the dentate area.

As mentioned above the usual dentate response to medial septal stimulation, *i. e.* the negative spike followed by a positive wave, was in some experiments at certain depths replaced by a single negative wave. The relation between the two types of septo-dentate response at various depths appears from Fig. 4. At a depth of 1.8 mm below the ventricular surface, corresponding to the upper blade of the granular layer, the response was of the ordinary type. At deeper levels the polarity of the positive wave was reversed. The pure negative wave recorded from the central and deeper parts of the dentate area had a longer latency than the negative spike recorded from the upper blade of the granular layer, as seen in the last record.

In conclusion, it may be stated that the septo-dentate response ordinarily consists of two components, an initial negative spike (representing the summation of several individual spike discharges) followed by a positive wave of longer duration. Sometimes the deeper parts of the dentate area may yield a response that consists only of a slow negative component. The initial spike and the subsequent wave — whether recorded positive or negative and isolated — are most likely due to the activity in two different structural elements within the dentate area. This assumption is further corroborated by the experiments to be described below.

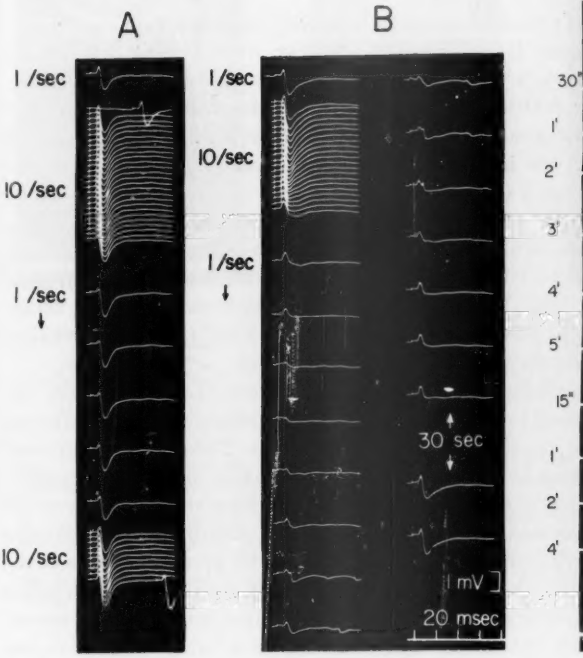

Fig. 5.

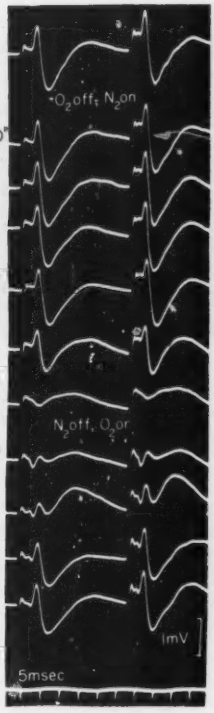

Fig. 6.

Fig. 5. *Excitability changes of the septo-dentate projection as tested by repetitive stimulation. A*, post-tetanic potentiation affecting the positive wave only. *B*, post-tetanic depression, after a series of tetani, influencing the positive wave to a greater extent than the initial spike.

Fig. 6. *Effects of anoxia on the septo-dentate response.* Paired shocks delivered to the medial septal area. The positive wave is reduced before the initial spike and is the last to appear on readmission of oxygen.

Excitability properties of the septo-dentate response. — The two components of the typical septo-dentate response behaved similarly when tested by paired shocks. The initial negative spike showed a moderate facilitation at delays from about 300 to 10 msec and was diminished at shorter delays. The absolute refractory period of this component measured 1.7—1.8 msec. The positive wave showed a similar facilitation and depression at the corresponding delays.

By repetitive stimulation the two main components of the septo-dentate response behaved differently. The amplitude of the initial spike was only little affected by stimulus frequencies up to 100/sec. In contrast, the following positive wave was often markedly depressed (Fig. 5 B) even at repetition rates as low as

5/sec. This depression was sometimes associated with an increase in duration of the initial spike.

Following a burst of repetitive stimuli of relatively short duration (5—15 sec) and moderate frequency (10—20/sec) the positive wave exhibited a distinct post-tetanic potentiation, whereas the initial spike was almost unchanged (Fig. 5 A). As typical for post-tetanic potentiation, its degree and duration was enhanced with increasing rate and duration of the tetanic stimulation, provided these values were not too high so as to produce depression. The post-tetanic potentiation lasted from 5 sec to 3 min.

More intense repetitive stimulation or a series of tetanic stimulation periods, resulted in a depression of all components of the septo-dentate response, lasting from a few seconds to 5—10 min. The initial spike was the most resistant part of the response (Fig. 5 B). The restitution of the septo-dentate response subsequent to a depression followed a typical sequence. First, the initial negative spike and then the positive wave gradually reappeared.

Spontaneous self-sustained discharges were seldom recorded from the dentate area as compared to the hippocampus. Those obtained from the dentate electrode consisted of positive and fairly slow waves. These were interpreted as discharges originating at some distance, most probably in the hippocampus. Simultaneous recording from the dentate area and from the field CA1 of the hippocampus corroborated this assumption. Also, afterdischarges following septal stimulation were more readily recorded from the pyramidal layer of the hippocampal field CA1 where they appeared as sharp negative waves, whereas the dentate electrode only recorded the distant, positive and slower potentials.

Effects of anoxia on the septo-dentate response. By anoxia a further distinction was made between the two main components of the septo-dentate response. The initial negative spike was more resistant to anoxia than was the positive wave (Fig. 6). The latter was sometimes replaced by a slow negative wave. In one experiment the spike was recorded for as long as 23 minutes during pure nitrogen breathing under over-pressure; its amplitude remained constant for about 4 minutes, followed by a gradual decrease. By readmission of oxygen full restitution took place within 2 minutes. The duration of the initial spike increased somewhat during the anoxic period. This was apparently due to the abolition of the subsequent positive wave. This demasking of the initial spike resembles the results obtained by repetitive stimulation (Fig. 5 B).

Relation between the activation of the dentate area by ipsilateral septal and by contralateral CA3 stimulation. The dentate response to stimulation of the septum and the contralateral field CA3 (the crossed CA3-dentate response) are shown in Fig. 1 and 7. The two potentials are similar in form. However, in spite of the longer conduction distance (as measured along the course of the ventral psalterium fibres), the latency of the crossed CA3-dentate response was considerably shorter than that of the septo-dentate response (Fig. 7). Furthermore, the amplitude of the crossed CA3-dentate potential was higher. By placing the

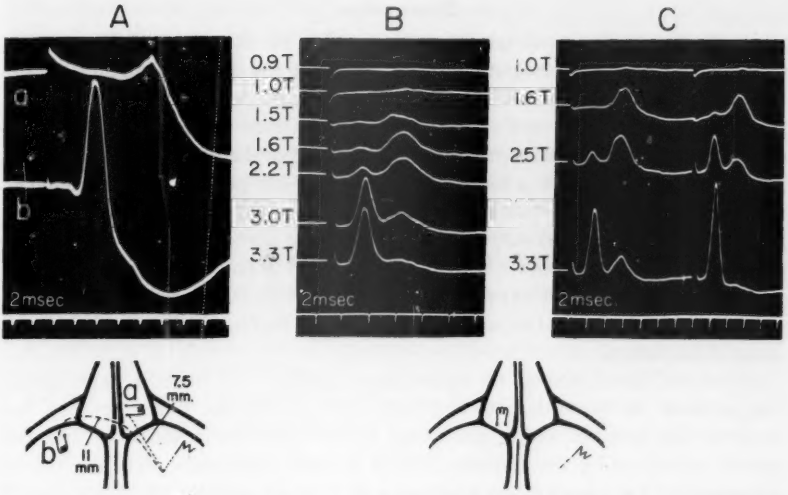

Fig. 7. *Relation between the septo-dentate and the crossed CA3-dentate response. A*, stimulation of ipsilateral medial septal region (a); stimulation of the contralateral CA3 (b). Recording from the same point within the dentate area. The conduction distances 7.5 and 11 mm respectively. — *B*, stimulation at increasing stimulus strengths (T = threshold) near the border between the septum and the hippocampus; recording from the contralateral dentate area. — *C*, electrode arrangement as in *B*. Paired shocks at a given interval with increasing intensities. Facilitation of the first, high-threshold spike of the CA3-dentate response and depression of the second, low-threshold spike of the septo-dentate response.

stimulating electrode on the caudal part of the septum, near the border to the hippocampal field CA3, the latter field could be excited by strong stimulation (Fig. 7 B and C). Weak stimulation (1—1.5T) elicited the negative spike of the septo-dentate response, which had a duration of 3.5 msec and a latency of 4.2 msec. By stronger stimulation (1.6 T) a new spike of 2.5 msec duration and a latency of only 1.8 msec appeared, probably due to spread of the stimulating current to the adjacent field CA3. Still stronger stimulation produced an augmentation of the short latency spike, whereas the late one was depressed and ultimately completely abolished. Since occlusion occurred when the stimulus strength was increased (Fig. 7 C), the two spikes probably represent the activity of the same elements evoked by afferent impulses through the two routes.

During anoxia the septo-dentate as well as the crossed CA3-dentate response showed within 1 or 2 min a depression of the late spike and the positive wave, whereas the initial spikes of both responses resisted anoxia for more than 10 min, although their amplitudes gradually diminished towards the end of this period. The high and similar resistance to anoxia of the initial spike of the two responses in question is also in favour of the assumption that the activation is a direct one for both afferent routes.

Discussion

Localization of the low-threshold structures within the septal region. The results presented indicate that the potential recorded from the dentate area on stimulating the septal region probably is due to the activation of neurons lying in the posterior, dorso-medial part of the septal nuclear mass on both sides, possibly the magnocellular medial septal nuclei. The fibres of the fornix and the ventral psalterium seem to have a higher threshold and are probably not stimulated directly by the weaker shocks. This interpretation of the origin of septo-fugal impulses coursing posteriorly into the fornix is in agreement with anatomical observations by METTLER (1943), ROSE and WOOLSEY (1943), DAITZ and POWELL (1954) and MCLARDY (1955 a). In these studies retrograde cell changes were found in the medial septal nucleus following damage to the hippocampus, fimbria or fornix.

Course and distribution of the septo-dentate impulses. The section experiments suggest that the septo-dentate impulses course along the fibres between the most medial bundles within the fornix system (the fornix superior) and the lateral ones forming the fimbria. This is in good agreement with the results presented by MCLARDY (1955 b) who stated that the medial fifth of the body of the fornix contained fibres that penetrated the corpus callosum to join the cingulum whereas the middle three fifths enclosed efferent fibres from the magnocellular part of the medial septal nucleus ending either in the hippocampus or in the temporal neocortex.

The recording from the various layers of the dentate area suggests that the septo-dentate projection activates two distinct zones, apparently corresponding to the two blades of the granule cell layer. There were additional signs of activation of cells lying in the hilus of the dentate fascia (see below). Stimulation of a single point within the posterior medial septal region caused a fairly widespread activation of the dentate granule cell layer. The experiments do not allow any conclusions regarding a possible topographical relationship between different parts of the medial septal nucleus and the various portions of the dentate area.

Mode of activation of the granule cells by septo-dentate impulses. The initial negative spike of the septo-dentate response probably represents discharges of the granule cells. It seems permissible to conclude that the single spike most likely is composed of the nearly synchronous discharges of a group of granule cells, whereas the multiple smaller spikes recorded with the micropipettes represent the firing of individual or of a small number of such cells. The short refractory period (1.7—1.8 msec) and the great resistance to anoxia suggest that the granule cells are excited monosynaptically by the septo-dentate path as previously indicated by EULER, GREEN and RICCI (1958).

The interpretation of the following slow wave is more difficult. This was recorded as a positive wave from the granule cell layer and usually also from the hilus fasciae dentatae (CA4). However, sometimes it was negative in the latter region. Thus, the wave may reflect the activity of two different elements.

One component of this wave may represent retrograde invasion of the depolarization from the soma into the dendritic tree of the granule cells, the other may be due to excitation of CA4 neurons. The depression of the positive wave by anoxia and by repetitive stimulation may be due to a block of this dendritic activation. The usual immense positivity of the slow wave when recorded from the hilus fasciae dentatae is also in favour of the interpretation of this component as partly due to spread of the excitation from the soma of the granule cells along their dendrites. The positivity and the high amplitude might be explained on the basis of the histological arrangement in the dentate area with the granular and molecular layers enclosing the hilus, through which the efferent axons of the granule cells, the mossy fibres, are coursing, an orientation resembling the closed field of LORENTE DE NÓ (1953).

In conclusion, the slow wave following the initial spike may be due partly to the activation of granule cell dendrites and partly to the excitation of CA4 neurons.

Relation between the ipsilateral septal and the crossed CA3 activation of the granule cells. The most striking feature when comparing these two routes of dentate activation is the similarity in form of the evoked potentials. The mutual occlusion of the negative spike indicates that the same elements are activated by the two afferents. Strong evidence is offered by experimental histological work that interhippocampal impulses activate the granule cells (BLACKSTAD 1956). This strengthens the assumption that the initial spike of the two responses represents the activity of granule cells. However, when comparing the two responses, it is evident that stimulation of the contralateral CA3 is the more effective one, producing a much larger response with a considerably shorter latency than that elicited by ipsilateral septal stimulation. The great amplitude of the spike evoked by contralateral CA3 stimulation indicates a high degree of efficiency of this afferent route. This is in agreement with the massive terminal degeneration around the proximal part of the granule cell dendrites following severance of the contralateral hippocampal formation (BLACKSTAD 1956). The shorter latency of the crossed CA3-dentate response suggests that the fibres mediating this response are thicker than those conveying the septo-dentate impulses.

The absence or scarcity of sustained electrical afterdischarges in the dentate area may possibly be explained on the basis of the different structural organization between this area and the hippocampus where such discharges are very common. The main difference seems to be the smaller possibilities for repetitive stimulation by way of recurrent collaterals within the dentate area.

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Acta physiol. scand. 1961. 51. 29—40

From the Neurophysiological Laboratory at the Anatomical Institute, University of Oslo, Norway

Activation of the Field CA1 of the Hippocampus by Septal Stimulation

By

PER ANDERSEN, HELGE BRULAND and BIRGER R. KAADA

Received 8 August 1960

Abstract

ANDERSEN, P., H. BRULAND and B. R. KAADA. *Activation of the field CA1 of the hippocampus by septal stimulation.* Acta physiol. scand. 1961. *51.* 29—40. — In rabbits under urethane-chloralose anesthesia the existence of a septo-hippocampal projection has been verified by the evoked potential method. Its origin is the magnocellular medial septal nucleus. These septo-fugal fibres activate the basal parts of the apical dendrites and/or the soma of the CA1 neurons, probably by a monosynaptic route. By repetitive stimulation and by strychnine the depolarized area of the CA1 neurons may increase, by anoxia it may decrease. Thus, according to the excitability level, a smaller or greater part of the apical dendrites may initiate spikes of an all-or-none character.

In a previous communication (ANDERSEN, BRULAND and KAADA 1960) the activation of the dentate area by stimulation of the medial septal nucleus was reported. The present article is concerned with the activation of the field CA1 of the hippocampus in response to stimulation of the same structure.

Material and Methods

Seventeen adult rabbits anesthetized with urethane-chloralose[1] were used. Detailed description of the technique has been given in a previous communication (ANDERSEN, BRULAND and KAADA 1960). In brief, the evoked potential technique was used, employing small stimulation and recording electrodes. The analysis of the responses were made by depth recording and stimulation, and by the use of paired and repetitive stimuli, anoxia and locally applied strychnine.

[1] The chloralose was kindly supplied by E. Merck, Darmstadt.

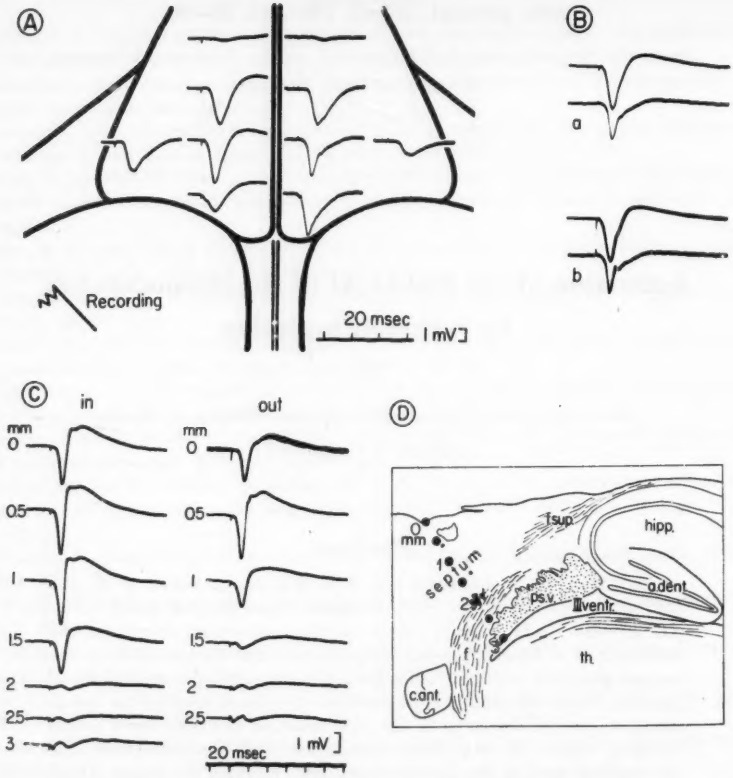

Fig. 1. *Origin of the septo-hippocampal projection. A.* Stimulation of various points on the dorsal surface of the septal region. The potentials recorded from the left hippocampus are transferred to the respective stimulation points. *B.* Records obtained (a) before and (b) after suction of the septum of one side (left). Upper beam ipsilateral (right), lower beam contralateral (left) records. *C.* Potentials obtained from the surface of CAl in response to stimulation at different depths as indicated in *D*. *In* shows records observed on insertion, *out* on retraction.

In all figures monopolar recordings and negativity upwards.

Abbreviations

a. dent.	dentate area	hipp.	hippocampus
c. ant.	anterior commissure	ps. v.	ventral psalterium
f.	fornix	th.	thalamus
f. sup.	superior fornix	III ventr.	third ventricle

Results

Stimulation of the exposed dorsal surface of the septum elicited a typical potential in the ipsi- and contralateral fields CAl of the two hippocampi. The response consisted of a positive/negative wave with one or two negative spikes superimposed on the positive wave (Fig. 1 and 2). The response thus resembles

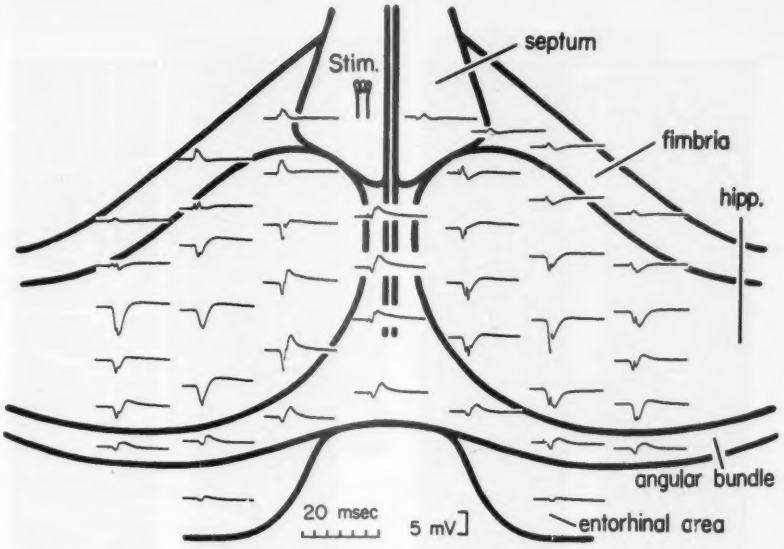

Fig. 2. *Distribution of the septo-hippocampal potentials.* The distribution is bilateral. Note the small potentials in CA3 (near the fimbria), over the angular bundle and in the entorhinal area.

the CA1 commissural potential evoked by stimulation of the symmetrical point of contralateral field CA1 (ANDERSEN 1959). The slow surface-positive and -negative waves had the same threshold, whereas that of the spikes was higher.

In order to delimit the structure within the septal region which gave rise to the hippocampal potentials, stimulation was performed at different surface points and at various depths through an electrode penetrating the septal nuclei, the fornix, and the ventral psalterium. The results are presented in Fig. 1. The excitable area was found to be situated in the postero-medial, dorsalmost part of the septum, corresponding to the magnocellular part of the medial septal nucleus (YOUNG 1936). No typical potential could be recorded when the stimulating electrode was placed within the fornix or the ventral psalterium. Thus, the projection in question appears to originate in the medial septal nucleus and represents a true septo-hippocampal pathway.

To determine whether the contralateral potential was a direct one or mediated via the septal nuclei of the opposite side, records were obtained bilaterally before and after suction of the septal nuclei of one side (Fig. 1 B). The upper record, in each pair is the ipsilateral response whereas the lower shows the contralateral response. The persistence of the potentials indicates that both projections are direct.

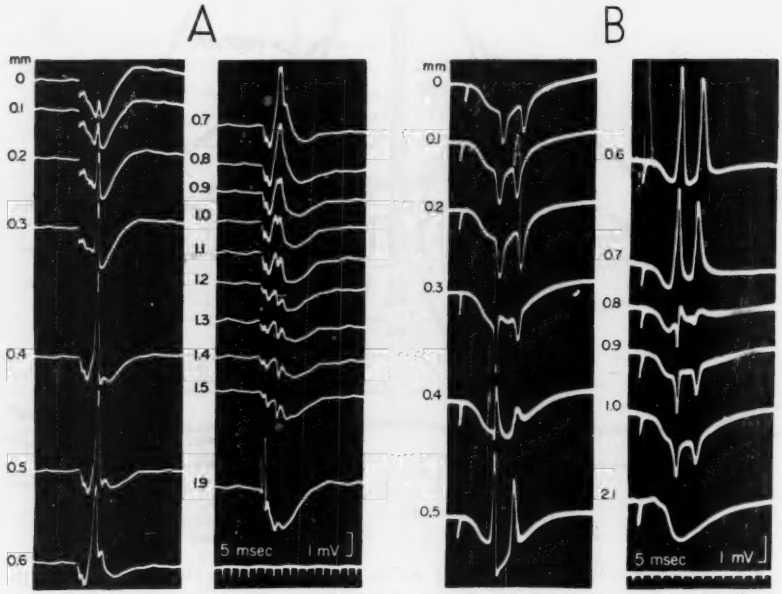

Fig. 3. *Depth recording.* Potentials obtained with glass capillary electrodes at indicated depths of CA1 on medial septal stimulation. *A* and *B* from two different experiments. Last records in *A* and *B* from dentate area for comparison.

Course of the septo-hippocampal pathway. Section of the fimbria and the exposed midline structures, including the fornix longus and the medial part of the fornix proper, were without effect on the typical CA1 potential. On the other hand, a lesion confined to the alveus, severing the white matter between the septo-hippocampal border and the recording electrode, abolished the response. It is concluded that the septo-hippocampal fibres are situated between the most medial and lateral parts of the fornix fibre system, and then course in the alveus almost directly to the dorsalmost part of CA1. Fibres to more lateral regions of this field probably follow the fimbria for a short distance before merging into the alveus.

Distribution of the septo-hippocampal projection. Surface recording. Stimulation of the postero-medial part of the septum elicited potentials distributed over a wide area of the dorsal hippocampus (Fig. 2). The area of maximal responses was found to be the CA1. Potentials of smaller amplitude were recorded from the CA3; their form was different from the typical CA1 response, being mainly surface negative. *Depth recording.* By this procedure the spikes were found to have their greatest amplitude in the pyramidal layer (0.4—0.5 mm) and in the adja-

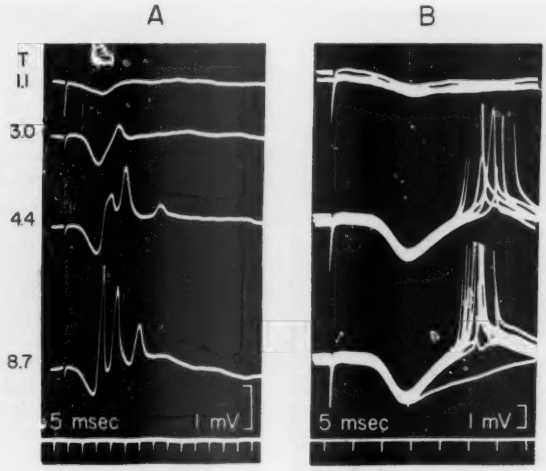

Fig. 4. *Relation between the deep negative wave and the spike. A.* Records obtained at 0.6 mm depth with increasing stimulus strength. T = threshold. *B.* Records obtained from 0.6 mm depth during restitution from a tetanus-produced depression. Further explanation in text.

cent part of the stratum radiatum (Fig. 3). In the latter region a substantial part of the surface-positive wave was recorded with reversed polarity. In some experiments, however, the positive wave was diminished but not reversed at 0.4—0.7 mm (Fig. 3 B). For reasons given below the deep negative wave is regarded as an excitatory postsynaptic potential elicited at the basal parts of the apical dendrites, suggesting that this part of the CA1 neurons is the main site of termination of the septo-hippocampal projection. Following single shock stimulation, negative spikes were not recorded from the rest of the stratum radiatum, suggesting that the spikes during such conditions are not conducted somato-fugally along the apical dendrites. This finding is in agreement with the results obtained by EULER, GREEN and RICCI (1958).

The relation between the spikes and the slow negative wave recorded from about 0.6 mm below the surface is elucidated by Fig. 4. *A* shows the effect of increasing stimulus strength. On increasing intensity the negative wave was augmented and one or more negative spikes occurred superimposed on it. The greater the negative wave the more spikes appeared. *B* shows three records taken during the restitution following a period of depression as a result of tetanic stimulation. The development of the negative wave (recorded from a depth of 0.6 mm) is associated with the appearance of spikes. These emerge from a given level of the negative wave, their latencies being shorter at the end of the restitution period as compared with the start.

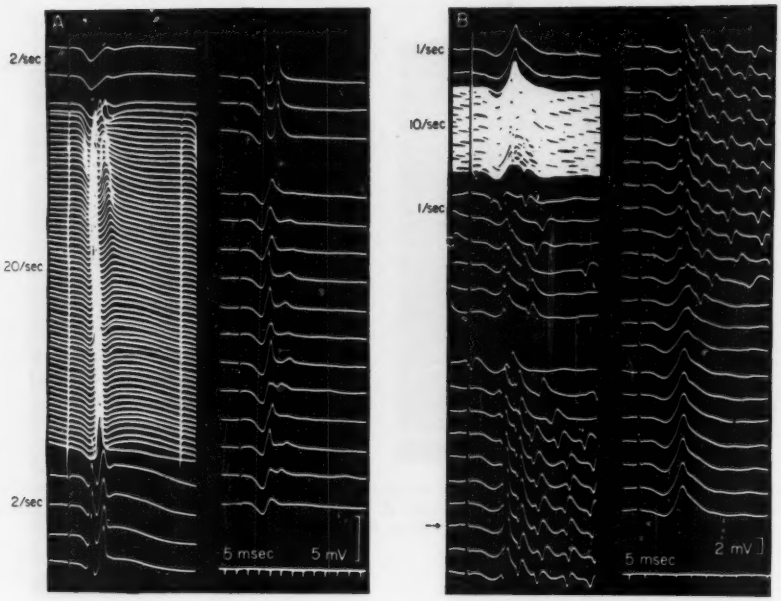

Fig. 5. *Effects of tetanic stimulation. A.* Surface CA1 records obtained at indicated stimulating frequencies. In right column the open space between the third and fourth sweep represents an interval of 18 sec (36 sweeps). Note change in latency of the spikes during and after the tetanic stimulation. — *B.* Records obtained 0.6 mm below the surface of field CA1 at indicated frequencies. Each record is shown, except that 5 sweeps (5 sec) have been omitted at the open space 7 sec after the tetanus. Repetitive discharges from about 12 sec after the tetanus. Arrow indicates the record which shows the purest negativity of the first spike of the repetitive discharges.

Excitability changes of the CA1 neurons produced by septal stimulation. Paired shocks delivered to the medial septal nucleus showed an enhancement of the test response, especially of the spike, at delays from about 10 to 200—300 msec. At delays shorter than 10 msec the test potential was diminished; total abolition occurred at a delay of about 4 msec, probably representing the absolute refractory period of the CA1 neurons activated by the septo-hippocampal impulses.

Tetanic septal stimulation caused several alterations of the CA1 response. These consisted of changes in the latency and in the amplitude of the spike, in the production of new spikes, or of afterdischarges.

In the experiment illustrated in Fig. 5A repetitive stimulation enhanced the amplitude of the first spike, and elicited a new spike after the first one. This second spike was much more susceptible to tetanic stimulation, being quickly abolished after the initial enhancement. During such stimulation the latency of the initial spike at first decreased; then it gradually increased and reached

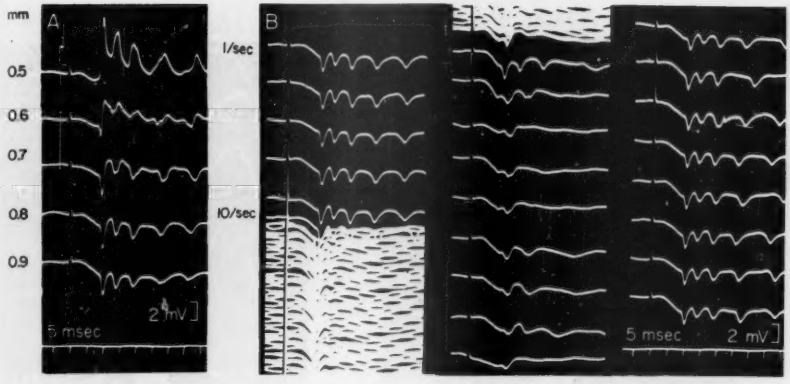

Fig. 6. *A.* Repetitive discharges recorded from indicated depths below the surface of CA1. *B.* Effect of tetanic stimulation on the repetitive discharges recorded 0.9 mm below the surface. Time interval between first and second columns of *B* is 10 sec. See text.

the prestimulatory value. The first single shock after the tetanus elicited a spike with a minimal latency and maximal amplitude, but after a while it gradually changed to the original form and time course. Principally similar results were observed with regard to the second spike.

Post-tetanic potentiation occurred almost regularly, affecting the spikes as well as the late surface-negative wave (Fig. 5A). The duration ranged from some seconds to about 3 min, depending upon the frequency and the duration of the tetanic stimulation. Occasionally a spike could be produced by post-tetanic potentiation. Excitation at a frequency above 30/sec often resulted in a pure depression of the potentials which outlasted the stimulation by several minutes. The first component to reappear was the surface-positive wave, followed by the surface-negative one; then the first and the second spikes returned.

In the period following a tetanus of relatively short duration or low frequency each single shock could elicit repetitive discharges (Fig. 5B and Fig. 6). These probably represent a sign of increased responsiveness of the CA1 cortex because this condition often preceded one in which tetanic stimulation produced the usual long-lasting and slow afterdischarges. The first spike of such repetitive discharges underwent a gradual change in configuration — when recorded from 0.6 mm below the surface (Fig. 5B). In the period immediately following the tetanic stimulation the repetitive discharges were irregular but after about 15 sec they became more uniform. The discharges consisted of diphasic spikes, first increasing, then decreasing in number. Concomitantly with the increased number of discharges the first spike changed from a positive/negative polarity to a pure negativity (at arrow) and subsequently resumed its original form. The

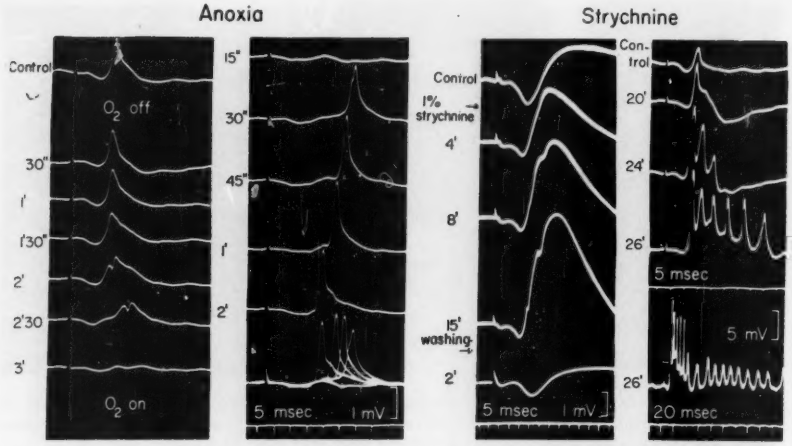

Fig. 7. *Effects of anoxia and strychnine on the septo-hippocampal potential. Anoxia.* Effect of $3\frac{1}{2}$ min anoxia on the potential recorded 0.7 mm below the surface of CAl. Note the change in spike polarity at 2 min. The second column shows the restitution with the augmented spike superimposed on a slow negative wave. In the last record the sweeps obtained during the restitution period are superimposed to demonstrate the relation between the slow negative wave and the spike. — *Strychnine.* Left column, surface records. Augmentation of surface negative wave with development of several spikes. Right column, records obtained 0.7 mm below the CAl surface. Strychnine applied at arrow. Increase of the deep negative wave and of amplitude and number of spikes. Last record obtained with slower time base to show the rhythmical and long-lasting discharge.

remaining spikes within the group were all positive/negative but the positivity was smallest when the first spike was purely negative.

Repetitive discharges recorded from different levels of CAl are illustrated in Fig. 6A. At the pyramidal layer (0.5 mm) the spikes are purely negative and superimposed on a slow negative wave. At deeper levels the spikes become diphasic, suggesting propagation of impulses from the cell bodies along the apical dendrites. At 0.9 mm the spikes are purely positive, suggesting a conduction block at this site.

Fig. 6B shows the effect of tetanic stimulation (10/sec) on the repetitive discharges recorded from the apical shaft layer (0.9 mm deep). The stimulation was followed by a period of depression lasting for about 20 seconds. At this low stimulating frequency no invasion of repetitive discharges into the apical dendrites was noted, as found by EULER et al. (1958). Higher frequencies produced such invasion, but from the layer of the terminal branches of the apical dendrites only positive potentials were recorded.

Effect of anoxia and strychnine. Deprivation of the oxygen supply resulted in the disappearance of the spike in 2—$2\frac{1}{2}$ min *pari passu* with a gradual and slow decrease of the negative wave recorded from a depth of 0.7 mm (Fig. 7). Before

the abolition of the spike its latency increased and the polarity shifted from pure negativity to positive/negative diphasicity, a phenomenon similar to that observed for the initial spike of the repetitive discharges (Fig. 5B). On readmission of the oxygen the spike occurred with increased amplitude and prolonged latency, superimposed on a slow negative wave. The further development was characterized by an augmentation of the negative wave and a decrease of the spike latency. This experiment shows that the spike initiating mechanism is ready for operation shortly after an anoxic period, and that the restitution of the mechanism for the triggering of the spike — probably the deep negative wave — is the essential factor in the establishment of a normal response.

Locally applied strychnine sulfate (a piece of filter paper soaked in a 1 per cent solution) produced the reverse of the anoxia effect (Fig. 7). The surface negative waves and the deep negative wave were markedly increased in magnitude as well as in duration and a series of negative spikes occurred superimposed upon them. Strychnine increased also the first spike (last column). When this was absent, strychnine could elicit a spike of the same latency as that of the usual first spike.

Discussion

The existence of a septo-hippocampal projection. As mentioned, the recorded hippocampal potentials are most likely due to excitation of the magnocellular medial septal nucleus. In addition, it cannot be excluded that the nucleus septo-hippocampalis (YOUNG 1936) contribute to the projection as well. The failure to produce potentials, with the same stimulus strengths, when the simulating electrode was situated within the fornix or the ventral psalterium, indicates that these fibre bundles have a higher threshold, and that excitation of those fibres plays a minor role, if any, in eliciting the recorded hippocampal potentials. Thus, the experiments suggest the existence of a true septo-hippocampal pathway, which originates bilaterally in the medial septal nuclei. Similar conclusions have been arrived at with regard to the septo-dentate projection (ANDERSEN *et al.* 1960). This interpretation is in essential agreement with studies on retrograde cell changes within the medial septal nucleus following destruction of the hippocampal formation (METTLER 1943, ROSE and WOLSEY 1943, DAITZ and POWELL 1954, MCLARDY 1955), and normal anatomical data (YOUNG 1936). According to METTLER, DAITZ and POWELL the projection is ipsilateral, whereas MCLARDY found degeneration on both sides following unilateral fornical section.

Several authors have described potentials recorded from the hippocampus in response to dorsal fornix stimulation (GREEN and ADEY 1956, DUNLOP 1957, EULER *et al.* 1958). In view of the high threshold of the fornix fibres compared with that of the medial septal nuclei it appears likely that they in fact have excited the latter nuclei. This would explain the surprisingly low chronaxie which GREEN and ADEY (1956) found by such stimulation. These authors state

that the low chronaxie suggests the presence of thick myelinated fibres within the fornix. However, morphological investigations have revealed very thin, myelinated nerve fibres only (SIMPSON 1952, DAITZ 1953). In two subsequent articles EULER and GREEN (1960 a, b,), using the same electrode arrangement as in their previous studies, state that they stimulate the septum and not the dorsal fornix fibres, thus expressing a view similar to ours.

Mode of activation of the CAl neurons by septo-hippocampal impulses. From the depth recordings it seems likely that the site of termination of the septo-hippocampal impulses is the pyramidal layer and the adjacent part of the apical shaft layer of CAl. The later part of the surface positive wave was usually recorded with the greatest negativity and steepest rising phase at this level and presumably represents the summated excitatory postsynaptic potential generated at the proximal part of the apical dendrites and on the soma of the CAl pyramidal cells. This interpretation is based on the polarity and form of the potential, its summation to a preceding similar potential at short intervals, and its probable causal relation to the spike formation (Fig. 4 and 7). A similar site of termination was found by EULER *et al.* (1958).

The absolute refractory period of the spike measured about 4 msec, suggesting that the pathway in question is a mono- or disynaptic one. Admittedly, the absolute refractory period of the septo-hippocampal response is longer than that of the septo-dentate response which measured 1.8 msec (ANDERSEN *et al.* 1960). Therefore, the possibility that the impulses to CAl are relayed through the dentate area must be considered. The difference in latency does not necessarily indicate a disynaptic transmission in the septo-hippocampal projection. There are no anatomical report which describes connections of the efferent fibres of the dentate granule cells — the mossy fibres — directly with CAl neurons. On the contrary, LORENTE DE NÓ (1934), in his delineation of the hippocampal fields, uses the line of disappearance of the mossy fibres as the border between the CA3 and CA2, the latter as well as CAl being devoid of them. If the septo-hippocampal impulses are relayed by the granule cells, the pathway must be a tri-synaptic one, a less likely possibility.

When the CAl pyramidal cells are activated by near-by, local stimulation, the absolute refractory period measures 1.5 msec (ANDERSEN and JANSEN 1961). Activation of the same neurons from the contralateral field CAl gives an absolute refractory period of 15 msec (ANDERSEN 1960). Thus, the corresponding value for septo-hippocampal activation, 4 msec, lies between these figures. The local stimulation activates the soma, while the commissural impulses excites the apical dendritic shafts. These observations precipitates the working hypothesis that the absolute refractory period of a neuron may be dependent upon which part is excited or, to put it another way: the absolute refractory period of the dendrites is possibly longer than that of the soma, accepting that these dendrites may conduct all-or-none impulses. The intermediate position of the absolute refractory period of the CA1 neurons in response to septal stimulation would then fit

with the assumption that the septo-hippocampal fibres terminate on the border between the soma and the apical dendritic shafts.

Excitability properties of the field CA1 as studied by the septo-hippocampal impulses. The ease with which electrical afterdischarges and post-tetanic potentiation may be produced within CA1 stands in a marked contrast to the conditions found in the dentate area (ANDERSEN *et al.* 1960). This difference may probably be explained by a different synaptical arrangement.

Of special interest is the peculiar variations in latency observed from CA1 during and after a period of tetanic septal stimulation (Fig. 5A). The increase of the spike latency after the initial shortening during the tetanus does not seem to be directly related to the mechanism responsible for the production of the spike since it was recorded with unaltered amplitude both during and after the tetanic stimulation. Therefore, the increase in the spike latency is probably due to a depression of the process leading to the spike discharge, the synaptic transmission, rather than the mechanism of spike discharge itself.

Some of the observations concerning the repetitive discharges deserve further comments. The temporary change in configuration of the first spike of the series of discharges from diphasicity to pure negativity (Fig. 5B) suggests that the spike took its origin at a certain distance from the electrode in the beginning of the period, whereas it started close to the electrode tip at the height of the period of increased excitability. The most probable site of the initiation of the spike by septo-hippocampal impulses under normal conditions is the cell body. In states of increased irritability, however, this area may increase and the spike can then take its origin from a greater area of the neuron, including part of the apical dendrites.

Conversely, anoxia was able to change a potential obtained from just below the pyramidal layer from negativity to positivity/negativity. This phenomenon could similarly be explained by a narrowing of the depolarized area during anoxia, from one comprising the soma and a part of the dendrites to one restricted to the soma alone.

The degree of excitability would then be directly related to the area of the neuronal cell membrane that the afferent impulses depolarize. The more the excitability is enhanced above the normal level, the greater is the part of the dendrites which may be invaded by the spikes initiated in the soma, as observed by EULER et al. (1958) and by EULER and GREEN (1960b). The repetitive spike discharges described above (Fig. 5B) may represent a transition between the normal condition and one of highly increased excitability resulting in prolonged electrical afterdischarges or in spontaneous, self-sustained epileptiform discharges.

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Does the Urinary Excretion of Imidazole Acetic Acid Reflect the Endogenous Histamine Metabolism in Man?

By

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Abstract

DUNÉR, H., S. O. LILJEDAHL and B. PERNOW. *Does the urinary excretion of imidazole acetic acid reflect the endogenous histamine metabolism in man?* Acta physiol. scand. 1961. *51.* 41—46. — The urinary excretion of free and conjugated histamine, histidine and imidazole acetic acid (ImAA) was studied in four healthy subjects and four patients with severe burns. ImAA was found to be a normal constituent of human urine. In the cases of burn, where the histamine excretion was increased during the first days after the accident, the ImAA excretion was normal and showed no significant variations. Administration of histamine or histidine gave a slight increase in the excretion of ImAA.

The catabolic pathways of histamine in vivo have, in the last few years, been clarified, particularly by the works of SCHAYER (1956) and TABOR (1956). The results have recently been reviewed by SCHAYER (1959). Studies with C^{14}-labelled histamine in man by SCHAYER and COOPER (1956) and NILSSON *et al.* (1959) have shown that the principal route in the histamine destruction was methylation to methylhistamine or its oxidation product methylimidazole acetic acid. The latter product constituted about 45 per cent of all C^{14}-labelled compounds found in the urine. A minor part of the histamine was inactivated by histaminase to imidazole acetic acid riboside (20 per cent) and imidazole acetic acid (10 per cent). 2—3 per cent was excreted as free histamine, which agrees with earlier results (ADAM 1950, DUNÉR and PERNOW 1958).

There are no known non-isotopic procedures for the determination of the major histamine metabolites in the urine, methylimidazole acetic acid and imidazole acetic acid riboside. Methods for isolation of imidazole acetic acid (ImAA) from urine have been described by HANSON (1957) and BALDRIDGE and TOURTELLOTTE (1958). Both methods employ adsorption on ion exchange resins and identification on paper chromatography by "diazo reagents". With this method HANSON (1957) found no ImAA in urine from normal human beings, while large amounts (650 and 2,600 μg per 24 hours) were extracted in two cases of urticaria pigmentosa. These patients are known to excrete large amounts of free histamine in the urine (BROGREN *et al.* 1958).

It is known from experiments on rats and dogs, that ImAA is formed also from histidine without histamine being involved (BALDRIDGE and TOURTELLOTTE 1958, LINDELL and SCHAYER 1958). In these animals the urinary excretion of ImAA is therefore no specific measure on the endogenous histamine release. However, since the daily histidine intake in man seems to be rather constant at an ordinary diet (BLOCK and BOLLING 1951), the urinary excretion of ImAA might be supposed to reflect changes in the histamine release. In order to study this hypothesis the effect of administration of histidine and histamine on the ImAA excretion was studied in healthy human subjects. The excretion was also studied in patients with severe burns, showing a high endogenous histamine liberation during the first days after the burn accident (BIRKE *et al.* 1957).

Material and Procedure

The urinary excretion of free and conjugated histamine, histidine and ImAA was studied in four healthy male subjects and three male patients with severe burns (20—40 per cent body area). The normal subjects were fed a standardized diet with a calculated histidine intake of 2—3 g per day.

The urine was collected in 24 hour periods. In the normals control samples were collected during 2—3 days. Then for two days 5 mg histamine base, diluted in 1,000 ml saline, was given each morning during four hours. After one or two control days 5 g of l-histidine was given by mouth at nine o'clock in the morning.

Analytical methods

Free and conjugated *histamine* were extracted and assayed as described earlier (DUNÉR and PERNOW 1956). All values refer to histamine base.

Histidine and ImAA. 50 ml urine was adjusted to pH 5 with a saturated solution of sodium carbonate, and concentrated to dryness in vacuo at 35—45° C. The residue obtained was dissolved in 3 ml of water. After centrifugation the supernatant was passed through a Dowex 1 column (1 × 40 cm), which had previously been washed with 2 N hydrochloric acid and water. The compounds were eluted with 1 N HCl. Fractions of 4 ml per 10 min were collected with an automatic fraction collector. Aliquots of 0.05 ml from fraction 1—10 were placed on filter papers (Whatman 1) and chromatographed in a one-dimensional descending system with butanol : acetic acid : water (40 : 10 : 10) as a solvent. Histidine and ImAA were used as reference substan-

Table I. Normal subjects

Case	Day	Procedure	Urine ml/24 hrs	Histamine μg/24 hrs		Imidazole acetic acid mg/24 hrs	Histidine mg/24 hrs
				Free	Conjugated		
1	1	—	800	6.4	17.6	3.4	131
	2	—	750	8.2	15.5	2.3	64
	3	Histamine 5 mg	1 750	184	—	4.3	100
	4	Histamine 5 mg	2 200	161	—	3.9	145
	5	—	1 100	25	53	3.2	131
	6	Histidine 5 g	1 800	19	28	3.6	198
	7	Histidine 5 g	1 500	15	24	5.0	173
2	1	—	690	13	15	0.8	211
	2	—	700	8.4	9	0.3	276
	3	Histamine 5 mg	1 400	110	45	0.7	312
	4	Histamine 5 mg	1 550	160	81	0.8	366
	5	—	700	50	24	0.6	—
	6	Histidine 5 g	800	7.2	12	1.3	339
	7	—	900	9.0	19	0.9	188
3	1	—	1 000	19	32	1.1	162
	2	—	600	15	21	0.8	—
	3	Histamine 5 mg	1 300	160	83	1.8	145
	4	Histamine 5 mg	1 600	214	121	2.4	165
	5	—	900	26	67	0.5	119
	6	Histidine 5 g	1 300	15	19	4.2	238
	7	Histidine 5 g	1 525	18	23	5.0	178
	8	—	1 100	17	20	0.9	—
4	1	—	600	16	16	1.7	164
	2	—	700	11	6	0.5	134
	3	Histamine 5 mg	1 400	52	72	2.9	183
	4	—	1 000	21	17	0.9	157
	5	Histamine 5 mg	1 500	63	51	—	168
	6	—	600	12	20	0.9	134
	7	—	700	8.3	13	1.1	143
	8	Histidine 5 g	1 100	14	10	19.8	197
	9	Histidine 5 g	1 000	19	21	7.0	226
	10	—	900	17	34	2.6	134

ces.[1] After drying, the chromatograms were sprayed with "diazo reagent" (sulfanilic acid followed by 10 per cent sodium carbonate solution). The coloured spots of histidine and ImAA were cut out and the colour eluted for one hour in 3 ml of tertiary butanol : water : 10 per cent sodium carbonate solution (25 : 25 : 5) (HANSON 1957). The colour intensity was read at 500 mμ in a Beckman DU spectrophotometer. As standards were used the histidine and ImAA references from the chromatograms treated in the same way.

Preliminary results gave a recovery of 83—87 per cent (five experiments) of ImAA added to normal urine.

[1] ImAA was synthesized and kindly put at our disposal by Recip, Stockholm.

Table II. Burns

Case	Day after burn accident	Urine ml/24 hrs	Histamine μg/24 hrs		Imidazole acetic acid mg/24hrs	Histidine mg/24 hrs
			Free	Conjugated		
1	1	750	80	49	2.6	113
	2	760	39	40	1.2	126
	3	730	16	22	2.9	139
	7	1 120	7.2	13	4.8	70
	8	760	11.3	23	3.4	66
2	1	1 600	58	93	4.8	44
	2	1 600	40	68	1.9	75
	3	1 500	9	45	3,1	84
	7	1 970	12	32	5.6	50
	8	2 390	14	21	7.3	95
	9	3 150	11	18	9.1	97
3	2	1 100	64	128	3.4	120
	3	1 320	28	73	4.3	140
	4	990	5	21	2.1	130
	5	1 050	11	24	4.1	270

Results

The results from the healthy subjects are given in Table I and from the burns in Table II.

Healthy subjects. The excretion of free and conjugated histamine during the first control periods varied within 6.4—19 μg per 24 hours and 6—32 μg per 24 hours respectively. An increase up to 214 μg per 24 hours and 121 μg per 24 hours respectively was obtained during the days of histamine administration. The increase in excretion of free histamine equalled 1.0—4.3 per cent of the infused amounts.

There was no significant change in the excretion of histamine during histidine administration when compared to control periods.

The amount of excreted ImAA during the first control periods was 0.3—3.4 mg per 24 hours. During the days of histamine administration the ImAA excretion was 0.7—4.3 mg per 24 hours and during histidine administration 1.3—19.8 mg per 24 hours.

The histidine excretion during the first control periods was 64—276 mg per 24 hours. During the days of histamine administration the histidine excretion was 100—366 mg per 24 hours and during histidine administration 173—339 mg per 24 hours.

Burns. The excretion of free and conjugated histamine during the first day after the burn accident was 58—80 μg per 24 hours and 49—128 μg per 24

hours respectively. There was a significant decrease in the excretion during the following days.

The excretion of ImAA was 2.6—4.8 mg per 24 hours during the first day after the burn accident and showed no clear tendency to change during the following days.

The excretion of histidine was 44—120 mg per 24 hours during the first day and there was no significant change during the rest of the observation time.

Conclusions

The values obtained for free and conjugated histamine in urine in the normal subjects during the control periods were within the normal ranges earlier reported (DUNÉR and PERNOW 1957). During intravenous infusion of histamine the urinary excretion of free histamine increased significantly. The percentage of infused histamine excreted as free histamine was of about the same magnitude as previously reported (ADAM 1950, DUNÉR and PERNOW 1958).

In the burn patients a significantly higher histamine excretion was noticed during the first day after the accident. The histamine values then decreased successively and were normal on the third day. These findings are in agreement with earlier observations (BIRKE *et al.* 1958).

By the method described it was possible to show that ImAA is a normal constituent of human urine. This is in contrast to earlier report, where ImAA is considered to be present only in cases with increased endogenous histamine release (HANSON 1957). The values obtained for histidine excretion were within earlier reported normal ranges (WOODSON 1948, ULRICH 1954).

The purpose of the present investigation was to determine if the ImAA excretion increased with histamine administration or in conditions of increased histamine release and thus could be used as a measure of the endogenous histamine metabolism. It was shown however, that ImAA in the urine increased only slightly after infusion of large amounts of histamine and that conditions with significantly increased endogenous liberation of histamine such as the initial stage of burn gave no raised excretion of ImAA. Even if the number of the examined cases are small the results seems to show that in a single case small or moderate changes in the endogenous histamine release are not significantly reflected in the excreted amount of ImAA. In other conditions, however, with even larger urinary excretion of free histamine a high output of ImAA has been observed (HANSON 1957). It was further shown, that the ImAA excretion also varies with the histidine intake, which earlier has been observed in animals (BALDRIDGE and TOURTELLOTTE 1958, LINDELL and SCHAYER 1958). It can therefore be concluded, that determination of ImAA in man can not be used clinically to estimate the endogenous histamine formation.

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Acta physiol. scand. 1961. 51. 47—61

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Transmission and Reflection of High Explosive Shock Waves in Bone

By

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Abstract

CLEMEDSON, C.-J. and A. JÖNSSON. *Transmission and reflection of high explosive shock waves in bone.* Acta physiol. scand. 1961. *51.* 47—61. — Various kinds of bone such as femur, ribs with intercostal muscles, sternum with ribs and intercostal muscles and spinal column from ox, and femur, and femur + tibia with intact stifle joint from horse were exposed to high explosive air shock waves in a detonation chamber. The patterns of the pressure wave in the bone caused by the shock wave were recorded piezoelectrically by means of a barium titanate pressure transducer. The transmitted wave consists of two parts. The first one, called the front pressure wave, is due entirely to the impact of the air shock wave on the front surface of the object. The second part is due mainly to the progressive loading of the static pressure in the air shock wave as it passes by the object. Due to the inhomogeneity of bone, the transmitted part of the incident shock wave is more or less changed since it is diffused and broken up by reflection and scattering. It is less changed in a long bone such as femur than in *e. g.* sternum or the spinal column. It is strongly changed by the passage between the two bones in a joint. Strong reflections occur at the bone surface. The velocity of the wave is about 3,500 m/sec. in the long bones. In the spinal column and sternum it is considerably lower and is dependent on the length of path covered.

When a sound wave or a finite pulse such as an elastic disturbance (pressure wave) caused by a high explosive shock wave propagates through a human or animal body, the wave will be more or less modified, since it is diffused and broken up by reflection and scattering due to its passing across a large number of boundary surfaces and regions of different density. This modification of the

incident shock waves in biological tissues is characteristic and represents the essential difference in the quality of shock waves in air and water on the one hand and the elastic disturbances caused by shock waves in the body on the other. The properties of shock waves produced by the impact of a high velocity missile and transmitted through muscle, liver, stomach, intestinal wall and human skull bone and beef ribs have been studied by HARVEY and MCMILLEN (1947) employing the spark shadowgram method. The heterogenous structure of these tissues caused a dispersion of the waves and made them appear as a series of wavelets. In a previous study (CLEMEDSON and PETTERSSON 1956), the pressure patterns in some body regions of rabbits exposed to high explosive blasts were recorded by means of a piezo-electric pressure transducer. The incident shock wave was found to be changed to a different extent in the various regions studied, the most pronounced changes occurring in the thorax.

The velocity of an air shock wave increases rapidly with increase in peak pressure. In water and in biological tissues, due to their low compressibility, the velocity of the shock wave produced elastic disturbance is almost unaffected by an increase in peak pressure. To a very good approximation, the velocity of such a disturbance in water and most biological tissues can be considered to be equal to the velocity of sound in water, *i. e.* about 1,500 m/sec. It can be assumed, however, that this does not apply to tissues containing air or gas *e. g.* the lungs, and to denser tissues such as cartilage and bone.

Since bone is the tissue in the body which has the greatest density, it would seem likely that the skeleton should influence a blast wave passing through the body more than any other structure, possibly with the exception of the lungs. The scope of the present investigation has been to study these problems with special reference to: 1. the propagation and attenuation of an elastic disturbance caused by a high explosive shock wave in osseous tissue, and 2. the effects of surrounding soft tissues on the reflection of such a disturbance against bone.

Materials and Methods

The specimens of bone used were: femur, ribs with intercostal muscles (part of the chest wall), sternum with unilaterally attached ribs and intercostal muscles and spinal column from ox and further femur and femur plus tibia with stifle joint from horse. With the exception of the intercostal muscles all musculature was carefully removed from the bone. In the specimen of femur + tibia, the muscles were carefully removed in order to keep the stifle joint intact. The specimens were exposed with the long axis of the bone parallel with the direction of propagation of the incident shock wave. The end surface of the bone turned against the charge was ground plane in order that the shock waves always should hit the bone surface strictly perpendicularly. In the case of ribs with intercostal muscles the direction of propagation of the shock wave was across the specimen.

The bone specimens were exposed to the shock waves in a detonation chamber described earlier (CLEMEDSON 1949, CLEMEDSON and CRIBORN 1955). The chamber was open in the distal end (c. f. CLEMEDSON and CRIBORN 1955), and therefore the shock wave produced was of short duration (overpressure phase about 3 msec). The charge employed

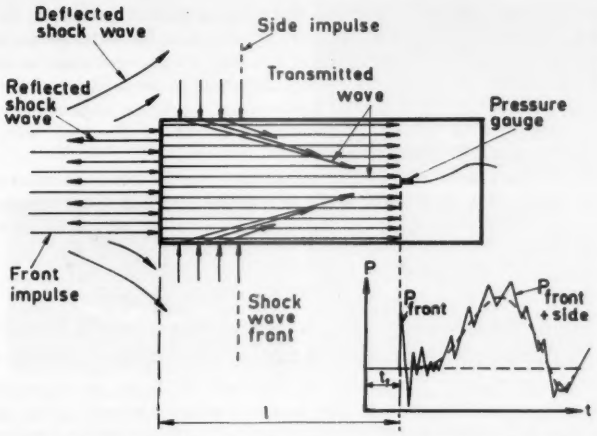

Fig. 1. Schematic diagram of phenomena accompanying the impact of a high explosive air shock wave on a biological medium. In the upper part of the figure is shown the incident, deflected and reflected parts of the shock wave and the generation of the transmitted waves. In the lower right part of the figure is shown the corresponding pressure-time diagram showing the two kinds of transmitted waves, *viz.* the front pressure wave and the static pressure wave. P_{front} and $P_{front + side}$ are the peak pressures of these two waves. t_1 is the time interval from the impact of the shock wave to the arrival of the front pressure wave.

was a plastic high explosive with the chief constituent being pentaerythritol tetranitrate (PETN). The weights of charge used were 2 and 4 g. The charge was fixed on the electric blasting cap (containing 0.2 g lead azide and lead trinitroresorcinate and 0.7 g trinitrotoluene) employed for ignition of the charge. The distance from the charge to the end surface of the bone specimen was 100 cm in all experiments, *i. e.* the plane surface of the bone was 54 cm from the opening of the chamber. This distance was used, because at that level of the chamber the shock wave has been found to be subject to minimum reflections and interferences from the walls and the opening. At that level in the chamber the peak static overpressure in the air shock wave is 2.4 kg/cm² and 3.8 kg/cm² for a 2 g and 4 g charge, respectively. The maximum reflection overpressure (against a plane, rigid wall perpendicular to the direction of propagation of the shock wave) is 7.9 kg/cm² and 15.2 kg/cm², respectively. The velocity of the air shock wave at that level is for the two weights of charge used about 580 m/sec and 680 m/sec, respectively.

The pressure transducers employed for recording of the shock wave patterns were the same type of barium titanate pressure transducer Model BC-10, manufactured by Atlantic Research Corp., Alexandria, Va., U. S. A., that have been used in a number of earlier physiological blast studies in our laboratory (Clemedson and Pettersson 1956, Clemedson *et al.* 1956, Clemedson 1956, Clemedson and Kulder 1959). Oscillations in the recording circuit are found which indicate a natural frequency of the gauge of about 210 kc. The capacitances of the gauges with their cables are about 0.007 μF, and when connected to the recording instrument, a Tektronix cathode ray oscilloscope 535, having an input resistance of 1 megohm, the time constants varied between 6 and 7 msec. Owing to the complexity of the recorded wave patterns and the relatively short durations of their most interesting parts, it has proved not to be necessary to use special amplifiers in order to increase the time constants.

The transducer was calibrated in air for shock waves passing parallel to its long axis as well as perpendicular to this axis. The tests showed that, for static pressures between 2 and 4 kg/cm², the transducers are in round numbers 15 per cent more sensitive when used with their long axes perpendicular to the direction of propagation of the air shock wave. The orientation of the gauge is, however, of much less importance when used in a medium such as bone tissue. The sensitivities of the gauges used were between 1.15 and 1.35 volts/kg/cm².

To enable the insertion of the pressure transducer into the bone specimen a hole was drilled in the bone. After insertion of the gauge, the hole was filled with molten gelatine which after coagulation assured a good contact between the transducer and the bone.

Results

A schematic representation of the pressure-time curve in a biological tissue exposed to a high explosive air shock wave is shown in Fig. 1. When a body of the kind considered here is exposed to the load from an air shock wave, the elastic disturbances set up inside the body at some distance from the area of application of the load, due to differences in velocity of propagation of the waves in air and in the body, are split up into two main parts (see Fig. 1). The first part is associated only with the impact of the air shock wave on the front surface of the object and consequently with a reflection pressure. The second part is due mainly to the progressive loading of the static pressure in the air shock wave as it passes by the object. The first part of such a disturbance in the body in question will from now on be called the front pressure wave and the second part the static pressure wave as this latter part in the recording points used is supposed to originate mainly from the load of the static pressure in the air shock wave. The front impulse caused by the incident air shock wave, which is a reflection impulse, is to a high degree dependent on the properties of the exposed medium.

When the air shock wave travels along in the detonation chamber and embraces the tissue specimen, the static pressure wave from the side surfaces successively reaches the pressure gauge delayed due to the lower velocity of the shock wave in air. The static pressure curve is rising only slowly due to the fact that this pressure wave is integrated of a very large number of impulses composed of parts with different path lengths in air and in tissue (see Fig. 1). The effect of the static load (side-on pressure) in a point in the object at some distance from the surface of application of the front impulse load probably reaches its maximum when the distance covered by the side impulse to the point in question is a minimum. Due to the finite length of the objects used in this investigation, very complex reflection phenomena are created, which influence the pressure wave pattern.

The appearance of the air shock wave generated in the detonation chamber is shown in Fig. 2. The leading part of an air shock wave *viz.* the transition zone in which the first pressure rise takes place is actually very small in thickness, probably of the order of 10^{-5} cm or just around the mean free path of the air

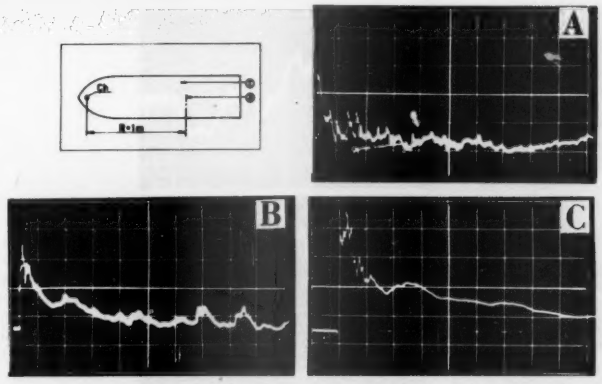

Fig. 2. Recordings of pressure pattern in the incident air shock wave. Weight of charge (*Ch*) used: A. and B. 2 g, C. 3 g. *Time* (between vertical lines): A. 1 msec, B. 200 μsec, and C. 20 μsec. *Pressure sensitivity* (between horizontal lines): 0.75 kg/cm² in all three recordings. The upper left picture is a schematic diagram showing the location of the pressure gauges (1 and 2) in the detonation chamber. Gauge 1 is used for synchronization of the sweep start of the oscilloscope with the arrival of the incident air shock wave front at the front surface of the specimen. Gauge 2 is used for recording of pressure pattern within the specimen.

molecules under normal conditions, corresponding to a rise time of the pressure of only a small fraction of a microsecond. This property of an air shock wave front can, however, not be expected to be truly reproduced by a pressure gauge of the type used in this study, since it has finite dimensions, and it, therefore, takes a certain time for the shock front to sweep over its surfaces (Cole 1948). Furthermore, the arrival of a shock front to the gauge sets up a disturbance within it, causing internal reflections. These phenomena are seen in Fig. 2 C. Due to the above-mentioned limitations of the gauge the steep shock front may, due to the sweep time used, be reproduced as a sloping line with small oscillations.

The following characters of the disturbances in the biological medium will be discussed, *viz.* the general wave pattern, the velocity of propagation, and the amplitude of the front pressure wave and of the static pressure wave.

General pressure wave pattern

A typical pressure wave pattern in a long bone (femur of ox) is shown in Fig. 3 A. The first, small peak to the far left in the curve is the front pressure wave, and the highest peak in the middle of the curve mainly corresponds to the maximum static pressure. The curve clearly demonstrates the complex pressure wave pattern described above. As the smaller oscillations in the curve have frequencies which are far from the resonance frequency of the gauge, it must be assumed that they mainly are due to reflection phenomena in the bone. It should be pointed out, however, that the reactions of this type of gauge, when introduced in such a medium as bone, is not known in detail.

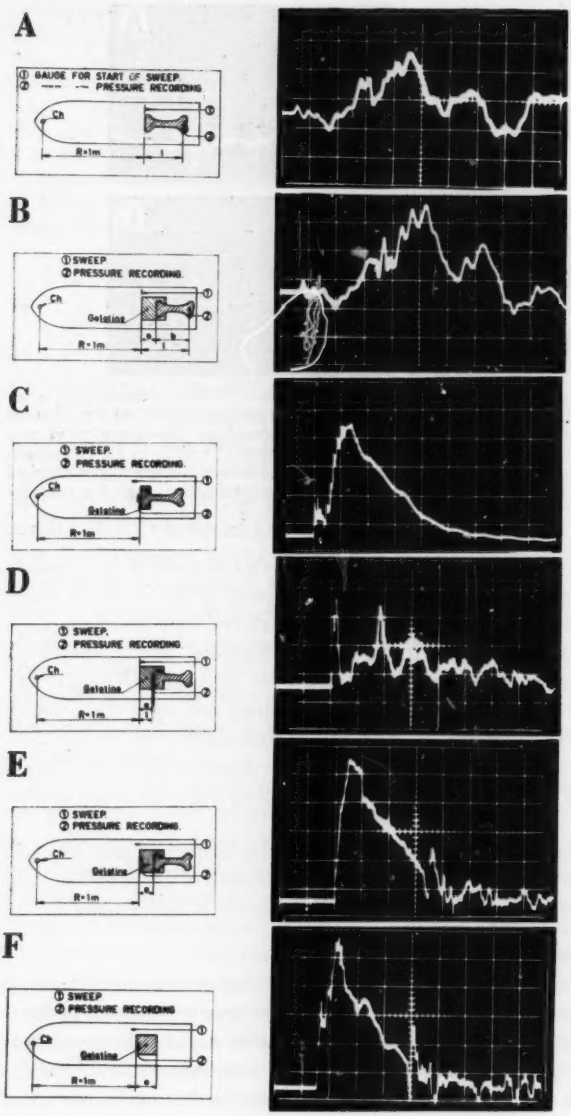

Fig. 3. Recordings of pressure patterns in an ox femur. The location of the pressure recording gauge is shown in the schematic diagrams in the left part of the figure. The small letters denote: *l* the distance covered by the shock wave from front of specimen to recording gauge; *a* distance from front surface of gelatine body to front surface of bone. Time, pressure sensitivity (between horizontal lines) and distance covered by the pressure wave in specimen are in:
A. 200 μsec, 0.4 kg/cm^2 and l = 310 mm;
B. 200 μsec, 0.4 kg/cm^2, a = 15 mm, b = 310 mm, l = 325 mm;
C. 50 μsec, 1.5 kg/cm^2;
D. 50 μsec, 1.5 kg/cm^2, l = 45 mm, a = 55 mm;
E. 50 μsec, 1.5 kg/cm^2, a = 140 mm; and
F. 50 μsec, 1.5 kg/cm^2, a = 140 mm.

Fig. 3 B is a similar recording from a bone to the front end of which had been cast a block of 20 per cent gelatine gel in order to simulate a body of soft tissue. The dimensions of the cylindrical block was: length 200 mm, and diam. 160 mm

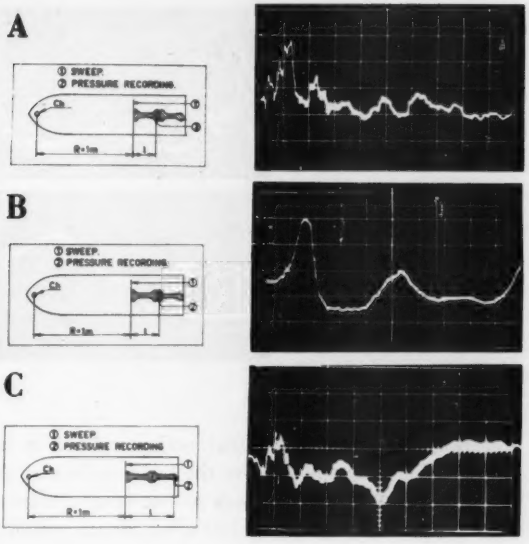

Fig. 4. Recordings of pressure patterns in a horse femur + tibia with intact stifle joint. Schematic diagrams in left part of figure show location of pressure gauge. Time, pressure sensitivity and distance covered by pressure wave in specimen are in:
A. 1 msec, 0.4 kg/cm², l = 325 mm;
B. 1 msec, 0.4 kg/cm², l = 410 mm; and
C. 2 msec, 0.08 kg/cm², l = 720 mm.

About 100 mm of the distal part of the bone was within the gelatine body. A comparison with Fig. 3 A shows that the pressure wave pattern is not significantly altered by a soft medium, which the wave has to pass when being transmitted from air to bone.

The shock wave patterns were essentially the same in the horse bone specimens.

In some experiments the bone marrow in the diaphyses had been removed. This did not significantly change the wave pattern.

A number of experiments have been performed in order to study the reflection of a shock wave against a bone surface. Fig. 3 C is from an experiment, in which the gauge was mounted directly against the flattened end surface of a femur. The disturbances in the rising part of the curve are caused by internal reflections inside the pressure gauge, when the incident shock wave hits the front side of it. The high peak is caused by the impulse reflected against the surface of the bone. The splitting up of the peak in a number of oscillations is in addition to internal reflections, probably also caused by a number of reflections back and forth between the bone and the rear side of the pressure sensitive part of the gauge.

The curve in Fig. 3 D was obtained with the gauge in the same position as in Fig. 3 C, but in this case a gelatine gel body had been cast on to the end of the bone. This will change the pressure pattern considerably. The first high pressure peak is caused by the incident shock wave hitting the gauge. The splitting up of this peak as seen in the previous curve (Fig. 3 C) is now almost quite absent. The second pressure peak appearing about 80 μsec later and having about

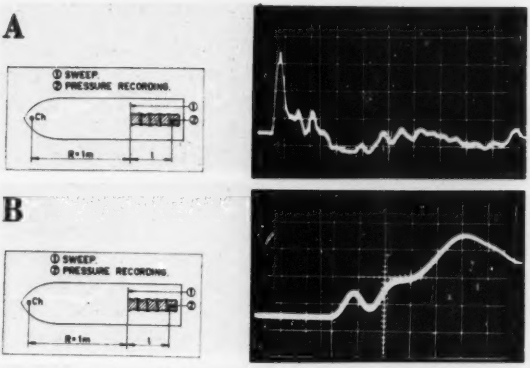

Fig. 5. Pressure recordings in a spinal column specimen from ox. Schematic diagrams showing location of pressure gauge in left part of figure. Time, pressure sensitivity and distance covered by pressure wave in specimen are in:
A. 1 msec, 0.4 kg/cm², l = 300 mm; and
B. 10 μsec, 0.08 kg/cm², l = 110 mm.

the same amplitude as the first peak is caused by the wave that has been reflected back from the bone to the front surface of the gelatine body and from there was again reflected back to the bone surface and the gauge. Since this wave has been reflected against a less dense medium (air), the pressure wave must have undergone a phase shift of 180° and consequently should have appeared as a downward deflection in the curve. This downward deflection is, however, almost immediately reverted by a new reflection against a denser medium (the bone surface).

When the gauge is located in the front surface of the gelatine body instead of at the surface of the bone, curves like the one in Fig. 3 E are obtained. In this case, the second peak, which is the pressure wave reflected back from the surface of the bone, is considerably smaller than the first peak. The large first peak in the curve corresponds to the maximum reflection pressure originating from the impact of the air shock wave on the front surface of the gelatine body. A comparison with Fig. 3 F, which is a recording of a shock wave pattern in a block of gelatine without the bone, and in which the reflection of the wave occurs in the boundary surface to the air instead of against the bone, shows that the patterns in the two cases are very much the same.

Some experiments were performed in which a specimen of horse femur and tibia with intact stifle joint was exposed to an air shock wave. The proximal end of the femur was facing the charge, and the pressure wave pattern was recorded at three different levels, *viz.* in the distal end of the femur (Fig. 4 A), in the proximal (Fig. 4 B) and the distal (Fig. 4 C) end of the tibia. It is evident, when comparing Figs. 4 A and 4 B, that the higher frequencies of the transmitted wave train are strongly damped by the passage through the joint. The completely different wave pattern in Fig. 4 C may partly be due to the fact that the recording gauge was located quite near the open end of the detonation chamber and was, therefore, probably influenced by reflections and interferences which

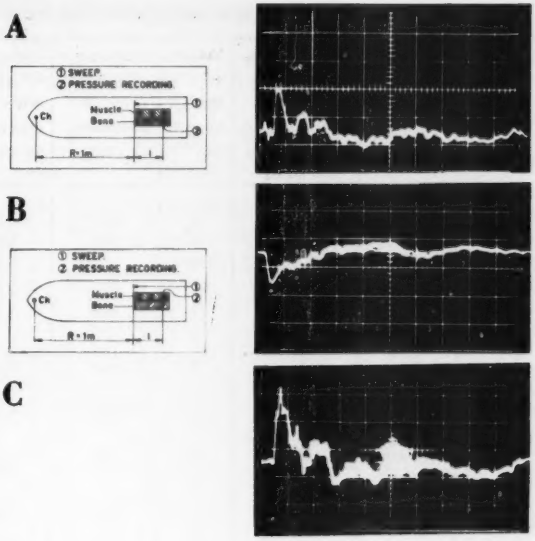

Fig. 6. Pressure recordings in sternum with unilaterally remaining ribs and intercostal musculature from ox. Location of pressure recording gauge: A. in sternum, B. in rib, C. in rib (sternum removed). Time, pressure sensitivity and distance covered by pressure wave in specimen are in:
A. 1 msec, 0.75 kg/cm², l = 180 mm;
B. 1 msec, 0.75 kg/cm², l = 130 mm; and
C. 1 msec, 0.4 kg/cm², l = 245 mm.

occur when an air shock wave is reflected at the open end of the detonation chamber. Consequently the shock wave load will be very complicated at that level.

The character of the pressure wave after passage through a part of the spinal column of an ox is shown in Fig. 5 A and 5 B. Fig. 5 A shows only the static pressure wave. Due to the low recording sensitivity in this case, the relatively small disturbance caused by the front impulse is not seen in this picture. This is instead shown in Fig. 5 B. The curves contain only few high freqency deflections probably due to the damping properties of the spinal column.

A pressure wave transmitted through the sternum is recorded in Fig. 6 A. Fig. 6 B was obtained from the same specimen, but in this case the gauge had been introduced into one of the ribs attached to the sternum, and the wave had to pass through a number of ribs and costal interspaces before reaching the gauge. The inverted form of the curve as compared with that in Fig. 6 A is enigmatic, especially as a shock wave passing through a similar specimen, in which the sternum had been removed, gave recordings like that in Fig. 6 C.

Velocity of pressure waves in bone

One of the main objects of this investigation was to determine the velocity of the front pressure wave in bone caused by a high explosive air shock wave. Due to the inhomogeneity of the bone tissue and the varying proportions of compact and spongious bone in different parts of the skeleton, it could be anticipated that the velocity of the transmitted wave should vary within rather wide

Table I. Data obtained in blast experiments on femur of ox and horse and femur + tibia of horse

Specimen	Number of experiments	Weight of charge in g	Pathlength of shock wave in specimen in mm	Velocity of shock wave in specimen in m/sec	Maximum pressure in kg/cm² in	
					Front pressure wave	Static pressure wave
Ox femur	9	2	310	3,960	0.14	0.9
Ox femur	2	2	300	3,200	0.07	0.7
Ox femur	2	4	300	3,130	0.12	1.0
Ox femur	2	2	276	3,640	0.11	0.9
Ox femur	2	4	276	3,610	0.15	1.3
Ox femur with marrow removed	2	2	300	3,300	0.06	0.6
Ox femur with marrow removed	2	4	300	3,190	0.1	1.0
Ox femur with marrow removed	2	2	276	3,840	0.06	1.6
Ox femur with marrow removed	2	4	276	3,590	0.1	2.8
Horse femur	6	2	354	3,470	0.04	0.2
Horse femur	4	2	315	3,370	0.07	0.6
Horse femur	2	2	300	3,660	0.02	0.8
Horse femur	2	4	300	3,540	0.03	1.1
Horse femur with marrow removed	2	2	315	3,290	0.04	0.6
Horse femur with marrow removed	2	4	315	3,200	0.06	0.7
Horse femur with marrow removed	2	2	300	3,610	0.015	0.5
Horse femur with marrow removed	2	4	300	3,510	0.02	0.7
Horse femur + tibia	3	2	748	3,120		0.12
Horse femur + tibia	2	4	748	3,300		0.13
Horse femur + tibia	4	2	410	3,100		0.59
Horse femur + tibia	1	4	410	3,110		0.89
Horse femur + tibia (gauge in femur)	2	2	325	3,490	0.06	0.85
Horse femur + tibia (gauge in femur)	1	4	325	3,320	0.1	1.45

limits. That this is the case is learnt from Tables I and II. In femur and tibia the velocity was found to exceed 3,000 m/sec in all experiments, and the main value obtained is 3,420 m/sec. The distance travelled by the wave within the bone specimen seems to have no influence on the speed of propagation, at least if the length of path does not exceed 750 mm. As seen from Table I, the velocity

Table II. Data obtained in blast experiments on spinal column, sternum and rib + intercostal muscles of ox

Specimen	Number of experiments	Weight of charge in g	Pathlength of shock wave in specimen in mm	Velocity of shock wave in specimen in m/sec	Maximum pressure in kg/cm² in	
					Front pressure wave	Static pressure wave
Spinal column of ox.....	3	2	312	2,230		2.1
Spinal column of ox.....	2	2	206	2,640		2.1
Spinal column of ox.....	2	2	108	3,240	0.06	2.4
Spinal column of ox.....	2	2	86	2,790		1.9
Spinal column of ox.....	2	2	53	2,870	0.18	2.7
Spinal column of ox.....	2	2	26	2,930	0.39	2.9
Spinal column of ox.....	2	4	312	2,100		3.1
Spinal column of ox.....	2	4	206	2,340		3.0
Spinal column of ox.....	2	4	86	2,400		3.6
Ox sternum	4	2	240	1,610	0.045	1.2
Ox sternum	2	2	180	1,900	0.28	1.3
Ox sternum	1	2	110	2,340	0.45	1.0
Ox sternum	1	2	47	3,360	0.8	1.7
Ox rib + intercostal muscles	3	2	200	[1](700)		0.9
Ox rib + intercostal muscles	3	2	130	[1](730)		1.0
Ox rib + intercostal muscles	2	2	67	(1,320)		1.0

[1] These low values are essentially derived from the velocity of the air shock wave.

of the pressure wave produced by a 4 g charge does not significantly differ from that of a wave produced by a 2 g charge, when the path covered is the same. The transmission of the wave seems to be mediated essentially through the compacta of the diaphyses, and removal of the bone marrow in the diaphysis does not influence the velocity of the pressure wave. In the spinal column, the mean velocity of the front pressure wave was 2,620 m/sec. In this case there was a tendency towards higher speeds of propagation with diminishing path length. In the sternum this tendency was strongly pronounced. In the specimen consisting of ribs with intercostal muscles very low velocities were found. Evidently, the first deflection recorded in these curves is not the front pressure wave but instead the static pressure wave. At least the two first velocity values in the rib + intercostal muscles specimen in Table II are essentially derived from the incident air shock wave and the deflection used for calculation of the velocity consequently is the static wave and not the front pressure wave, which has probably been too small to be recorded. This is due to the fact that the exposed front surface, *i. e.* the end area of the spinal column, is too small and that the inhomogeneous structure of the column exerts a rather strong damping effect.

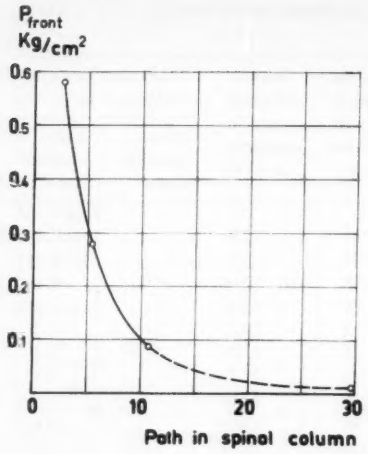

Fig. 7. Diagram showing the peak pressure in the front pressure wave as a function of the distance covered by the wave in a spinal column specimen from ox.

Pressure measurements in the transmitted waves

The maximum pressures in the front pressure wave and in the static pressure wave are found in Tables I and II. The maximum pressure in the static pressure wave was found to be 5 to 20 times larger than that in the front pressure wave in the long bones, and up to 40 times larger in the spinal column. The amplitude of the front pressure wave at various levels in the spinal column was especially studied in one series of experiments. As seen in Fig. 7 it diminishes with increasing length of path covered, essentially due to the damping properties of the inhomogeneity of the spinal column.

Some experiments were made in order to try to detect an increase in pressure due to reflection against the bone surface. Specimens of 20 per cent gelatine

Table III. Location of pressure transducer in reflection experiments

Experimental set up according to Fig.	Number of experiments	Path length in gelatine block in mm	Maximum pressure in first positive pressure peak in kg/cm²
3 E, but gauge 140 mm from front surface....	6	130—140	3.3
3 F	9	130—135	6.0
3 E, but gauge 65 mm from front surface....	3	60— 65	3.6
3 F	10	50— 60	5.3
3 E	3	130—140	9.3
3 D	5	130—140	9.2
3 C	3	0	4.5

gel only and of gelatine gel cast on the proximal end of ox femurs were exposed to shock waves. The locations of the pressure transducer in the various experiments are shown in Table III and Fig. 3 C—3 F. The weight of charge used was 2 g in all experiments. The means of the pressure values obtained in these experiments are found in Table III. It is seen that the pressure in the gelatine body is increased by 50 to 100 per cent due to reflection against the surface of the bone. When the pressure gauge is located in the front surface of the gelatine body (Fig. 3 F) instead of at the front surface of the bone (Fig. 3 D) no such increase in pressure is obtained.

Discussion

The present investigation has shown that the elastic disturbance caused by the incident air shock wave is heavily influenced and changed when passing through various types of bone. When the incident shock wave hits the bone surface — the same also applies for other kinds of tissue — a pressure wave corresponding to the front impulse is propagated through the bone. When the air shock wave embraces the bone this furthermore experiences a static impulse, which is propagated to an arbitrary point within the bone as a very complex train of waves, which are integrated to a more long-lasting static pressure wave. The front pressure amplitude is rapidly damped, and at some distance from the front surface of the specimen the amplitude of the static pressure wave may be 5 to 20 times larger than that of the front pressure wave. The front pressure wave is very much dependent on the physical properties of the medium through which it is propagated. The amplitude of this wave is also dependent on the size of the front surface of the exposed medium.

The degree of reflection and transmission of a sound wave or a finite pulse passing through various body tissues is dependent on the physical properties of the tissues. One factor of importance is the density of the medium, which multiplied by the velocity of the wave in the medium gives its wave resistance or acoustical impedance. At a boundary surface between two media of different density, a part of the wave in the primary medium is reflected, the rest being transmitted to the secondary medium. The greater the difference in acoustical impedance between two adjacent media (*e. g.* units of tissue) the greater part of the wave will be reflected back into the primary medium at the boundary surface. The tissue in the body, which has the greatest density and hence the greatest acoustical impedance, is the bone tissue. A complex pattern of reflection and transmission may be expected in the body. It has been shown in the present investigation that strong reflections occur at the boundary surfaces between air and tissue (gelatine gel) and against bone surfaces. The strong decrease of pressure in the pressure wave with increasing distance travelled by the shock wave in the spinal column is also an expression of that.

When an elastic disturbance propagates through a tissue, there is a certain loss of energy to the particles of the medium. In a liquid, the loss of intensity over a travelled distance (d) is

$$I = I_o e^{-2\alpha d},$$

where I_o is the incident intensity, and α the absorption coefficient of the traversed medium. The insignificant decrease in pressure with increasing length of path in the femur as compared with that in the spinal column seems to indicate that the reduction due to reflection and dispersion plays a greater role than the energy loss due to form and volume changes in the medium in reducing the amplitude of the pressure wave.

A sound wave propagating through a medium travels with a velocity (c_o) which is characteristic for that medium. It is dependent on the density of the medium according to the following equations:

in liquids: $c_o = \sqrt{\frac{1}{K \cdot \varrho_o}}$,

and

in solids: $c_o = \sqrt{\frac{E}{\varrho_o}}$,

in which c_o = sound velocity, ϱ_o = density at rest, K = compressibility and E = module of elasticity. As shown by these formulas the properties of density and elasticity is of greatest importance for the propagation of the disturbances discussed here.

For a shock wave, things are much more complicated. According to the Rankine-Hugoniot shock wave equations (*c. f.* PENNY and PIKE 1950) the shock front velocity (u) is

$$u = v_o \left[(p_1 - p_o)\ (v_o - v_1) \right]^{1/2}$$

if v_o and p_o are volume and pressure on the low pressure side and v_1 and p_1 the corresponding factors on the high pressure side.
If $p_1 \to p_o$ one will find that $u \to c_o$, or in other words, at infinitesimally low overpressure the wave velocity approaches that of sound.

FRUCHT has determined the velocity of sound in various soft body tissues. In fat tissue the velocity was found to be about 1,450 m/sec, in brain and kidney about 1,560 m/sec, and in spleen, liver and muscle about 1,580 m/sec. The velocity of a high explosive pressure wave in muscular tissue has been found to be of the same order of magnitude (CLEMEDSON and JÖNSSON). It has been demonstrated in the present investigation that the velocity of pressure waves in long bones is about 3,500 m/sec within rather wide limits. In the spinal column the velocity is considerably lower. It is evident that the lower velocity is due to the composition of the spinal column with alternating bone tissue and softer intervertebral discs. It should also be pointed out in this connection that the velocity of elastic disturbances in bone may differ in various directions of propagation due to structural differences.

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Observations on the Effect of Ethanol on the Urinary Excretion of Histamine, 5-Hydroxyindole Acetic Acid, Catecholamines and 17-Hydroxycorticosteroids in Man

By

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Abstract

PERMAN, E. S. *Observations on the effect of ethanol on the urinary excretion of histamine, 5-hydroxyindole acetic acid, catecholamines and 17-hydroxycorticosteroids in man.* Acta physiol. scand. 1961. *51.* 62—67. — In healthy young men the urinary excretion of histamine and 5-hydroxyindole acetic acid did not indicate that ethanol in moderate doses (0.5—0.7 g/kg) releases histamine or serotonin in the organism. This makes it unlikely that flushing produced by moderate ethanol doses is associated with the release of one of these substances. The adrenaline excretion after ethanol (0.3—0.4 g/kg) indicated a modest and short-lasting effect on the adrenal medullary secretion. The significance of this effect is discussed. No evidence for a concomitant change in the secretion from the adrenal cortex after ethanol (0.5—0.7 g/kg) was obtained.

It is generally recognized that many of the symptoms produced by ethanol in man are due primarily to disturbances of the motor and sensory functions of the central nervous system. There are, however, symptoms which are less readily explained by such mechanisms. One of these is the cutaneous vasodilatation seen already after moderate amounts of ethanol, the so-called "flush". Flushing of the skin is known to occur under conditions which favour a release of histamine or serotonin in the body. To find out whether ethanol in

moderate amounts affects the release of these substances, the urinary excretion of histamine and of 5-hydroxyindole acetic acid, the principal breakdown product of serotonin, were studied in man after administration of ethanol in the present investigation. More pronounced changes in the release of histamine and serotonin should be detected in this way.

Studies dealing with similar problems have been made by others. DRAGSTEDT *et al.* (1940) reported that perfusion of the guinea pig lung with a solution containing ethanol caused liberation of a histamine-like substance into the perfusate, and they suggested that ethanol stimulates gastric secretion via histamine. On the other hand SCHACHTER (1952) found no release of histamine from cat skin when perfusing with an ethanol solution. TROQUET (1958) found no histamine release from various rat tissues *in vitro* after ethanol. In man HOLLER, NEUGEBAUER and SCHMID (1950) noted a slight increase in blood histamine after small ethanol doses.

An interesting finding by SNOW *et al.* (1955) is that flushing, as a sign of serotonin release, could be provoked by small amounts of ethanol in some patients with carcinoid tumours. Recently, ROSENFELD (1960) has reported that a narcotic ethanol dose (4.5 g/kg) exerts an appreciable inhibitory effect on the metabolism of injected serotonin in mice. In the same report he also mentions that the urinary 5-hydroxyindole acetic acid excretion was decreased in human subjects during a 5-hour period following ingestion of 1—2 g/kg ethanol, but does not give any further data about these experiments.

Recent studies on the urinary catecholamine excretion in man (PERMAN 1958, ABELIN, HERREN and BERLI 1958) have indicated that increased secretion from the adrenal medulla occurs after moderate amounts of ethanol (< 1 g/kg). The magnitude and duration of this effect was also studied in the present investigation. Urinary 17-hydroxycorticosteroids have also been measured in similar experiments in order to study any concomitant change in the secretion from the adrenal cortex. It has been shown by several workers (SMITH 1950, 1951, FORBES and DUNCAN 1951, SANTISTEBAN and SWINYARD 1956, and others) that in various animals severe, acute ethanol intoxication (3—9 g/kg) is associated with an increase in the activity of the adrenal cortex. KRUSIUS, VARTIA and FORSANDER (1958) did, however, not find any significant change in plasma 17-hydroxycorticosteroids or in the eosinophile count in man after a moderate dose of ethanol (< 1 g/kg).

Material and Methods

Seven healthy young men, all with a history of very modest ethanol consumption, served as test subjects. The experiments were started at 09.00 after a light morning meal. No alcohol intake was permitted during 24 hours preceding the experiment. Smoking was not permitted during the experiment. The ethanol was given as whisky (43 per cent by volume) with the intake spaced over a period of 20 min. Urine was collected in periods of 2 or 3 hours.

Table I. Diuresis and urinary excretion of histamine, 5-hydroxyindole acetic acid and 17-hydroxycorticosteroids in man in a 3-hour period following ethanol 0.5—0.7 g/kg and in control experiment

C = control experiment
E = ethanol »
M. ± S. E. = mean ± standard error

Subject No.	Diuresis ml/min		Histamine μg/hour		5-hydroxyindole acetic acid μg/hour		17-hydroxycorticosteroids mg/hour	
	C	E	C	E	C	E	C	E
1	0.53	2.05	1.12	0.95	204	254	0.58	0.69
2	0.83	2.56	0.57	1.02	270	282	0.47	0.38
3	0.53	0.64	1.08	0.21	189	190	0.35	0.35
4	0.83	1.75	0.50	0.48	234	205	0.74	0.93
5	0.78	0.90	0.39	0.22	310	210	0.36	0.41
6	1.03	1.10	0.81	0.56	242	212	0.49	0.66
7	0.72	2.50	0.28	0.46	216	243	0.52	0.55
M. ± S. E.	0.75 ± 0.063	1.59 ± 0.227	0.68 ± 0.011	0.56 ± 0.012	238 ± 15.5	228 ± 12.5	0.50 ± 0.049	0.57 ± 0.079

I. Histamine, 5-hydroxyindole acetic acid and 17-hydroxycorticosteroids

The test subjects were studied in one ethanol and one control experiment. Both experiments were carried out under similar conditions on different days. Urine was collected in two periods: 09.00—11.00 and 11.00—14.00. In the ethanol experiment 120 ml whisky (0.5—0.7 g/kg) was given at the beginning of the second period. In the control experiment a corresponding amount of water was given. Otherwise no food or drink was allowed during the experiment. The diuresis during the first period 09.00—11.00 was noted. The urine collected during the second period was analyzed for histamine, 5-hydroxyindole acetic acid and 17-hydroxycorticosteroids. Histamine was determined according to the method of DUNÉR and PERNOW (1956) and 5-hydroxyindole acetic acid according to the method of MACFARLANE *et al.* (1956). 17-hydroxycorticosteroids were determined according to the method of SILBER and PORTER (1954).

II. Catecholamines

Three of the test subjects were studied in one ethanol and one control experiment. Urine was collected in the following periods: 09.00—11.00, 11.00—13.00 and 13.00—15.00. In the ethanol experiment 80 ml whisky (0.3—0.4 g/kg) was given at the beginning of the second period. During the third period the test subjects were allowed to eat and drink. Determinations of adrenaline and noradrenaline were made according to the method of EULER and LISHAJKO (1959). Excretion figures are given in ng per minute in terms of the hydrochlorides of the amines. Blood ethanol concentrations were determined with the enzymatic method of BONNICHSEN and LUNDGREN (1957) in double samples.

Table II. Urinary excretion of adrenaline and noradrenaline before and after administration of ethanol (0.3—0.4 g/kg) and in control experiment. Ethanol is given at 11.00—11.20. Figures are mean values in a group of 3 subjects

	Adrenaline ng/min			Noradrenaline ng/min		
	09.00—11.00	11.00—13.00	13.00—15.00	09.00—11.00	11.00—13.00	13.00—15.00
Control experiment	8.9	8.4	5.8	25	29	25
Ethanol experiment	8.3	14	5.8	25	26	34

Results

Table I shows the diuresis and the urinary excretion of histamine, 5-hydroxyindole acetic acid and 17-hydroxycorticosteroids in the ethanol and control experiments. The urinary excretion of the substances is not consistently changed by ethanol administration and seems to be independent of the diuresis level. The well-known effect of ethanol on the diuresis is marked in some of the subjects. (The diuresis during the first period, 09.00—11.00, was similar in the two experiments.)

In table II mean values from the catecholamine experiments are given. In these experiments the peak blood ethanol levels were 0.036—0.046 per cent. The adrenaline output increased only in the first 2-hour period after ethanol administration. No concomitant effect on the noradrenaline output was observed.

Discussion

The results seem to permit the conclusion that moderate ethanol intake causes no major changes in the endogenous release of histamine or serotonin. This makes it unlikely that flushing or other ethanol effects are produced by such mechanisms.

Increased adrenaline excretion was found after moderate ethanol doses. This is in general agreement with previous results obtained in man (PERMAN 1958, ABELIN *et al.* 1958) which have suggested that ethanol increases the secretion from the adrenal medulla. The present results indicate that the increased adrenaline release caused by ethanol is of moderate magnitude and short duration. The lack of effect on the other biogenic amines studied here makes it unlikely that this effect is unspecific. The excretion pattern of catecholamines is of the same type as that seen in hypoglycemia (EULER and LUFT 1952). However, FORSANDER, VARTIA and KRUSIUS (1958) and others have found no blood sugar fall in man during the first 2 hours after a similar ethanol dose (< 1 g/kg). In the anaesthetized cat, where ethanol also provokes an increased adrenal medullary secretion (PERMAN 1960), no consistent changes

in the blood sugar were noted within 1 hour after ethanol in doses of < 2 g/kg (PERMAN, unpublished observations). Therefore is seems unlikely that the adrenaline release after ethanol serves the glycemic homeostasis. It does, however, seem likely that ethanol increases the adrenaline secretion via nervous pathways and that the effect is short-lasting and of moderate magnitude, but its pharmacodynamic significance is not clear at present. Possibly the increased adrenal medullary secretion is associated with the general stimulating action noted in man after small amounts of ethanol. Ethanol is considered to depress the inhibitory functions of the cerebral cortex early. Results of electrical stimulation in the orbital cortex suggest that this area normally inhibits the secretion from the adrenal medulla via the hypothalamus (EULER and FOLKOW 1958). Ethanol, acting on the cortex, might relieve hypothalamic centers from this inhibiting influence, thus causing increased adrenal medullary secretion. The urinary excretion of 17-hydroxycorticosteroids was mainly unchanged after ethanol which suggests that the adrenal cortex is not concomitantly affected. This is in agreement with the results of KRUSIUS *et al.* (1958).

The author expresses his thanks to Dr. H. DUNÉR and Dr. B. PERNOW who performed the histamine and 5-hydroxyindole acetic acid determinations at the King Gustav V Research Institute, Stockholm, and to Dr. B. HÖKFELT, Department of Endocrinology, Karolinska Sjukhuset, Stockholm, for the 17-hydroxycorticosteroid determinations.

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Acta physiol. scand. 1961. 51. 68—74

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Effect of Ethanol and Hydration on the Urinary Excretion of Adrenaline and Noradrenaline and on the Blood Sugar of Rats

By

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Abstract

PERMAN, E. S. *Effect of ethanol and hydration on the urinary excretion of adrenaline and noradrenaline and on the blood sugar of rats.* Acta physiol. scand. 1961. *51.* 68—74. — Evidence that ethanol increases the adrenal medullary secretion has previously been obtained from studies of the urinary catecholamine output in man and other studies. — In rats moderate ethanol doses (2.4—2.7 g/kg) increased diuresis and noradrenaline excretion in comparison with similarly hydrated controls (6.1—6.8 ml/kg). However, further data suggest that ethanol effects on the catecholamine excretion in the dose-range 0.7—3 g/kg are explained by the diuretic action of ethanol. Hydration experiments showed that the noradrenaline excretion increased with rising diuresis. The adrenaline excretion was increased at moderate hydration levels. — Ethanol doses above 4 g/kg produced increased adrenaline output and hyperglycemia, as reported by others, and concomitantly caused major changes in the general condition of the rat. It is suggested that the effect of high ethanol doses on the adrenal medullary secretion is secondary to anoxia, due to hypoventilation.

In dogs with acute sublethal ethanol intoxication KLINGMAN and GOODALL (1957) found increased urinary excretion of catecholamines. In other reports (KLINGMAN and HAAG 1958, KLINGMAN, BANE and HAAG 1958, KLINGMAN, HAAG and BANE 1959) strong indirect evidence was presented that ethanol in lethal or sublethal doses markedly increases the activity of the sympathetic nervous system, including the adrenal medulla. PERMAN (1960 a) found increased amounts of catecholamines in the adrenal vein blood of anesthetized cats given ethanol.

In man studies on the urinary catecholamine output have indicated that moderate ethanol doses increase the adrenal medullary secretion (PERMAN 1958, ABELIN, HERREN and BERLI 1958). This effect is probably short-lasting and of small magnitude (PERMAN 1960 b). The urinary catecholamine output after ethanol, mainly in a low dose-range, was studied in rats in the present investigation. Previously, CRAWFORD and LAW (1958) and others have used the adrenaline and noradrenaline excretion of the rat as indicator of alterations in the activity within the sympathetic nervous system induced by various drugs. Recently, WARTBURG, BERLI and AEBI (1960) have reported that an acute ethanol dose (1.2 g/kg) increases the urinary excretion of adrenaline and noradrenaline in rats during a 24-hour period, and that chronic ethanol administration decreases the catecholamine excretion.

Ethanol administration involves administration of considerable fluid volume, and ethanol has in itself a marked diuretic action. It was therefore necessary to use hydrated rats as controls in the present investigation, and also to study the catecholamine excretion in rats hydrated to different degrees to see if the excretion is influenced hereby.

The effects of ethanol on the blood sugar in various species have been reviewed by TENNENT (1941). It has been found that ethanol doses above 3 g/kg generally cause hyperglycemia, whereas lower doses are without effect on the blood sugar. KLINGMAN and co-workers (KLINGMAN and BANE 1955, KLINGMAN, HAAG and BANE 1958) presented evidence that this hyperglycemia is secondary to an increased secretion from the adrenal medulla. The effect of low and high ethanol doses on the blood sugar and on the general condition of the rats was also studied in the present investigation. In man the blood sugar is mainly unaffected or decreased by moderate ($<$ 1 g/kg) ethanol doses (FORSANDER, VARTIA and KRUSIUS 1958, and others).

Material and methods

Rats of the Sprague-Dawley strain, body weight 250—350 g, were used in all experiments. They were maintained on a standard stock diet between experiments.

The experiments were performed between 09.00 and 15.00. Groups of 5 rats were used in the urinary excretion experiments. Each animal in the group received the same amount of fluid and ethanol. The dose was calculated from the total group weight, which gives only a minor error as the individual weights varied little within each group. After administration by stomach tube the animals were kept in metabolic cages for a period of five hours without access to food and water. It had previously been established that the diuresis produced by ethanol or by hydration occurs within 3—4 hours after administration and that the diuresis level is almost back at the resting level after 5 hours. The urine was collected in cylinders containing 2-N H_2SO_4 for a final pH of 2—3, and did not come in contact with metal in the collection system as this would influence the catecholamine recovery. After filtration on a suction funnel, catecholamines were determined according to the method of EULER and LISHAJKO (1959). Preliminary experiments had shown that in this way satisfactory excretion values could be obtained.

Table I. Effect of hydration on diuresis and urinary excretion of adrenaline and noradrenaline in rats. The statistical significance of the increase in mean values with increasing hydration, when compared to the lowest hydration level (6.4—7.6 ml/kg), is expressed with the following symbols:

° : $p > 0.05$
$^{+}$: $0.05 > p > 0.01$
$^{++}$: $0.01 > p > 0.001$
$^{+++}$: $0.001 > p$
M. ± S. E. = mean ± standard error

Number of experiments	Hydration ml/kg	Diuresis ml/kg/hour M. ± S. E.	Adrenaline ng/kg/hour M. ± S. E.	Noradrenaline ng/kg/hour M. ± S. E.
19	6.4— 7.6	1.3 ± 0.07	17 ± 2.3	51 ± 3.2
25	12.8—15.0	2.2 ± 0.08^{+++}	30 ± 2.2^{+++}	73 ± 2.8^{+++}
12	18.3—21.6	3.1 ± 0.12^{+++}	32 ± 5.2^{+}	72 ± 4.3^{+++}
10	22.5—25.5	4.2 ± 0.16^{+++}	20 ± 2.5°	76 ± 5.7^{+++}
4	31.0—31.6	5.3 ± 0.40^{+++}	14 ± 2.0°	81 ± 10 $^{++}$

Table II. Effect of one ethanol dose (2.4—3.0 g/kg) at 2 different hydration levels on diuresis and urinary excretion of adrenaline and noradrenaline in rats. The statistical significance of the increase in mean values after ethanol in the 2 groups is expressed with the following symbols:

° : $p > 0.05$
$^{++}$: $0.01 > p > 0.001$
M. ± S. E. = mean ± standard error

Number of experiments	Hydration ml/kg	Ethanol g/kg	Diuresis ml/kg/hour M. ± S. E.	Adrenaline ng/kg/hour M. ± S. E.	Noradrenaline ng/kg/hour M. ± S. E.
19	6.4— 7.6	0	1.3 ± 0.07	17 ± 23	51 ± 3.2
8	6.1— 6.8	2.4—2.7	1.9 ± 0.18^{++}	22 ± 4.9°	92 ± 11^{++}
25	12.8—15.0	0	2.2 ± 0.08	30 ± 2.2	73 ± 2.8
5	12.9—15.0	2.6—3.0	2.7 ± 0.27°	23 ± 6.7°	87 ± 10°

Table III. Effect of ethanol (0.7—3.0 g/kg) on the urinary excretion of adrenaline and noradrenaline in rats compared to a hydrated control group

M. ± S. E. = mean ± standard error

Number of experiments	Ethanol g/kg	Diuresis ml/kg/hour M. ± S. E.	Adrenaline ng/kg/hour M. ± S. E.	Noradrenaline ng/kg/hour M. ± S. E.
25 (hydrated controls)	0	2.2 ± 0.08	30 ± 2.2	73 ± 2.8
5	0.7	1.9 ± 0.19	33 ± 4.9	58 ± 6.7
12	1.3—1.4	2.1 ± 0.17	22 ± 4.0	71 ± 5.5
13	2.4—3.0	2.2 ± 0.19	26 ± 4.7	92 ± 7.8

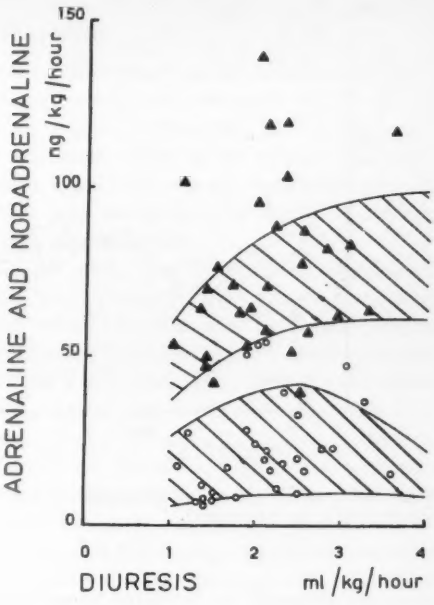

Fig. 1. Urinary excretion of adrenaline and noradrenaline related to diuresis in 30 ethanol experiments (0.7—3.0 g/kg). The range of values obtained in hydration experiments without ethanol are denoted by striped areas.

○ = adrenaline
▲ = noradrenaline

In the blood sugar experiments all animals received the same amount of fluid, 25 ml/kg. Blood samples were taken from the tip of the tail and the determination method of Hagedorn and Jensen was used. The general condition of the animals was roughly assessed by inspection and by handling.

Tap water at room temperature was used in the hydration experiments. Ethanol solutions were made up with tap water. Dilutions of 5, 10, 20, 30 and 40 % (v/v) were used, the 40 % solution when doses above 3 g/kg were administered. When lower doses were given, different dilutions were used at each dose level.

Results

The results of the urinary excretion experiments are found in Table I—III. Table I shows that when the diuresis was increased by hydration, the urinary noradrenaline excretion rose to a higher level and remained elevated with a tendency to increase further with increasing diuresis. The adrenaline output was somewhat increased at moderate hydration levels (12.8—15.0 ml/kg).

Table II shows that ethanol, 2.4—2.7 g/kg, produced increased diuresis and noradrenaline excretion when given with a moderate fluid volume (6.1—6.8 ml/kg) and compared with controls receiving a corresponding water volume. However, the same ethanol dose had no effect on the noradrenaline excretion if diluted to a larger volume (12.9—15 ml/kg) and compared with similarly

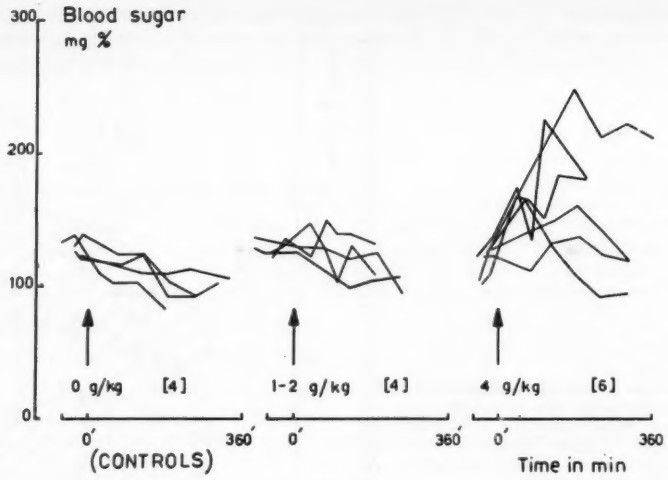

Fig. 2. Effect of ethanol on the blood sugar of rats. Figures within brackets denote number of experiments at each dose-level.

hydrated controls. In the latter experiment diuresis levels in the 2 groups did not differ significantly. These findings suggest that here the ethanol effect on the urinary catecholamine output is due to the diuresis produced by ethanol. Table III shows that 3 ethanol doses in the range 0.7—3 g/kg have no significant effect on the urinary catecholamine excretion when compared to controls in a similar diuresis range. Fig. 1 shows the catecholamine excretion related to the diuresis in the individual ethanol (0.7—3.0 g/kg) experiments in comparison to the range of values obtained in control experiments with similar diuresis induced by hydration. The rise in noradrenaline output with increasing diuresis can be seen and also that the ethanol experiments do not differ consistently from the control experiments.

In 2 experiments with ethanol in doses of 4 and 5.7 g/kg the adrenaline excretion was strongly increased (80 and 130 ng/kg /hour) whereas the noradrenaline excretion was in the normal range ($<$ 100 ng/kg/hour).

The blood sugar (Fig. 2) was mainly unaffected by ethanol in a low dose range (1—2 g/kg) while a dose of 4 g/kg produced hyperglycemia. The general condition of the rats in these experiments was not affected by hydration or by ethanol doses below 3 g/kg. With doses above 4 g/kg gross signs of ethanol intoxication with varying degree of anesthesia regularly appeared. As judged by the colour of the blood samples taken for sugar determination, the animals were in a state of hypoxia at these dose levels. A dose of 6 g/kg was lethal for some of the rats.

Discussion

On the basis of experimental data on the urinary catecholamine excretion in man under various conditions it has generally been assumed that the diuresis can vary over a considerable range without influencing the catecholamine excretion. PITKÄNEN (1956), in a systematic study of the urinary adrenaline output of rats under various conditions, reported that it was largely independent of the diuresis induced by hydration. The catecholamine output at different diuresis levels has apparently not been studied further.

The present results, obtained in rats, show that the noradrenaline output increases with a rise in the diuresis level, although the changes noted were small. The fact that increased diuresis induced by ethanol or by hydration gave similar changes seems to rule out an effect of hydration in itself, because ethanol did not affect the catecholamine output. Under the same conditions the adrenaline output was increased at moderate hydration levels only. The reason for the different behaviour of the two catecholamines is not clear at present. That the diuretic effect of ethanol did not always appear is not surprising because "ethanol diuresis" and "water diuresis" are produced by the same mechanism, namely inhibition of the release of antidiuretic hormone (VAN DYKE and AMES 1951, and others). If therefore the amount of fluid administered causes a strong "water diuresis" ethanol does not produce an additional effect (Table II).

Ethanol in the dose-range 0.7—3.0 g/kg did neither overtly affect the general condition, nor the blood sugar level or catecholamine output of the rats. Thus, no evidence for an effect of moderate ethanol doses on the adrenal medullary secretion, as noted in man under similar conditions, was obtained in the rat. As the effect in man is probably moderate and short-lasting it is possible that the 5-hour collection period used here did not permit its detection, but marked changes in the output can be excluded. Ethanol doses above 4 g/kg produced increased adrenaline output and hyperglycemia, which is in agreement with the findings made by previous workers. The fact that these effects appear at dose levels where ethanol greatly affects the general condition of the rat suggests the possibility that they are secondary to anoxia which is known to produce similar changes via a strong stimulation of the sympathetic nervous system. That ethanol, as well as other substances with narcotic properties, produces a respiratory depression in a high dose-range is well known from clinical experience. KLINGMAN and HAAG (1958), using the respiratory rate as indicator of the respiratory function, noted that the primary cause of death in ethanol intoxication was respiratory failure in approximately 65 % of their dog experiments.

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Acta physiol. scand. 1961. 51. 75—83

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Effect of Reserpine on the Storage of Catechol Amines in Brain and Other Tissues

By

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Abstract

BERTLER, Å. *Effect of reserpine on the storage of catechol amines in brain and other tissues.* Acta physiol. scand. 1961. *51.* 75—83. — The effect of reserpine on the metabolism of dopamine and noradrenaline in tissues has been studied. Dopamine was found to be depleted from the brain at a higher rate than noradrenaline; dopamine decreased to 50 per cent of the normal level in 15 min while the corresponding time interval for noradrenaline was 45 min. The dopamine in the peripheral tissues of the sheep was found to be only slightly lowered within 13 hours, after administration of 2 and 4 mg reserpine per kg whereas the dopamine of the brains were reduced to insignificant amounts. In this respect it behaved in the same way as 5-hydroxytryptamine (5-HT). The rate of disappearance of catechol amines after reserpine is suggested to be dependent on the rate of turnover of the amines. The principal effect of reserpine on the tissue catechol amines and 5-HT is supposed to be due to an interaction of the active transport of the amines into the storage sites. The results of the investigation indicate that the drug does not interfere with the decarboxylation of dihydroxyphenylalanine and 5-hydroxytryptophan.

In 1955 SHORE, SILVER and BRODIE showed that reserpine released 5-hydroxytryptamine (5-HT) from its body stores (see SHORE et al. 1957). A similar effect of the drug on the tissue catechol amines was shown shortly afterwards by CARLSSON and HILLARP (1956) who demonstrated that the drug caused adrenaline to disappear from the rabbit's adrenal medulla. BERTLER, CARLSSON and ROSENGREN (1956) found a complete depletion of noradrenaline from the rabbit's

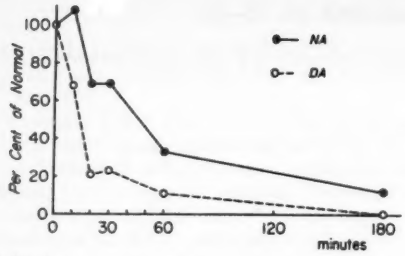

Fig. 1.

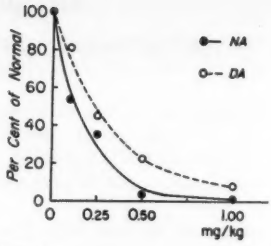

Fig. 2.

Fig. 1. Noradrenaline and dopamine in rabbit brain at various times after reserpine (1 mg per kg body weight).

Fig. 2. Effect of various doses of reserpine on noradrenaline and dopamine in rabbit brain. Time interval 16 hr.

heart and HOLZBAUER and VOGT (1956) observed a marked decrease of the noradrenaline content in the hypothalamus of the cat after administration of reserpine. The data concerning 5-HT indicate that reserpine acts by impairing the storage of this amine (SHORE et al. 1955, CARLSSON, SHORE and BRODIE 1957). The effect of reserpine on catechol amines seems to be an impairment of storage mechanisms, too (CARLSSON et al. 1957), but it is possible that this is not the only effect of the alkaloid.

This paper gives an account of some different lines, which have been tried in order to further elucidate the mechanism of reserpine action. The investigation is not finished, but had advanced so far, that the results may be discussed. Of the catechol amines studied, special attention is attached to dopamine (3-hydroxytyramine) for the following reasons: 1) It is less studied than noradrenaline and adrenaline. 2) It offers special interest because of its strict localization to the corpus striatum of the brain. 3) It probably constitutes the precursor of noradrenaline.

Table I. Effect of reserpine on the catechol amines and 5-hydroxytryptamine in various tissues of the sheep

μg/g

	Control			Reserpinized		
	NA	DA	5-HT	NA	DA	5-HT
Corpus striatum.....	0.1	5.6	0.6	0.0	0.0	0.1
Lung	0.1	3.0	1.6	0.0	1.6	0.6
Heart	2.2	0.2	—	0.0	0.2	—
Spleen.....	5.7	0.4	—	0.0	0.1	—
Duodenum.....	0.1	1.3	14	0.0	0.4	3.3

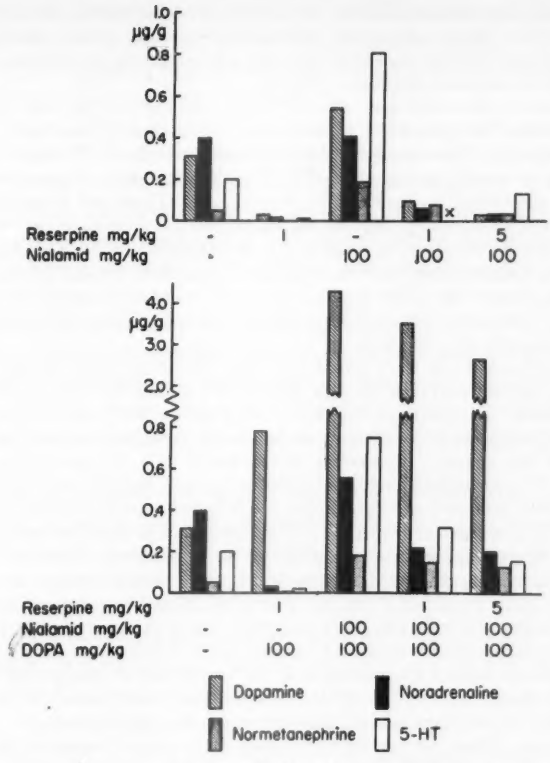

Fig. 3. Effect of drugs on noradrenaline, dopamine, normetanephrine and 5-hydroxytryptamine content in rabbit brain.
Time intervals: Reserpine 16 hr. Nialamide 6 hr. DOPA 30 min.

Experimental

Effect of reserpine on dopamine and noradrenaline in rabbit brain: Reserpine in a dose of 1 mg per kg body weight was given i.v. to adult male rabbits. The animals were killed by a blow on the neck at varying intervals of time after the injection. The brains were immediately dissected out and examined for their catechol amine contents (see below). The results are given in Fig. 1. The effect of varying doses of reserpine on the dopamine and noradrenaline contents in rabbit brain was studied in another set of experiments (Fig. 2).

Effect of reserpine on catechol amine and 5-HT contents in various sheep tissues: In order to investigate the effect of reserpine on the dopamine stored in the chromaffin cells of ruminants (FALCK, HILLARP and TORP 1959, BERTLER *et al.* 1959) reserpine in a dose of 2 mg and 4 mg per kg was given subcutaneously to two sheep, weighing about 25 kg. The animals were killed about 13 hours after the injection of reserpine. Two sheep of the same weight served as controls (Table I).

Influence of monoamine oxidase inhibitors and DOPA on catechol amines and 5-HT in rabbit brain: Rabbits were given intravenous injections of the very potent and long-acting monoamine oxidase inhibitor nialamide after the administration of reserpine. The doses and time intervals employed are seen in Fig. 3.

D,L-3,4-dihydroxyphenylalanine (D,L-DOPA) (100 mg per kg) was administered i.v. to rabbits which had previously been treated with reserpine, nialamide or reserpine followed by nialamide. The animals were killed 30 min after the DOPA injection (Fig. 3).

Determinations of catechol amines and 5-HT: The determination of noradrenaline and dopamine was performed as described by BERTLER, CARLSSON and ROSENGREN (1958) and CARLSSON and WALDECK (1958). Normetanephrine was determined according to the method described by BERTLER, CARLSSON and ROSENGREN (1959). The application of this method for tissue extracts will be published elsewhere. Estimation of 5-HT was carried out fluorimetrically after purification on a column of cation exchange resin (BERTLER and ROSENGREN 1959). The details of this procedure which have not been published earlier, will be given here.

Before the preparation of the column, the resin (AMBERLITE XE-64) has first to be freed from the smallest particles. For this purpose the resin is slired up in dilute sodium hydroxide solution. The particles which do not settle in 3—5 min are decanted off. This procedure is repeated 15—20 times and then the resin has a suitable mesh-size for preparation of the column (dimension in H^+-form: 6 × 25 mm). The column is washed with 2 N hydrochloric acid, redistilled water and, immediately before use, with 20 ml of N sodium acetate-acetic acid buffer, pH 6.5, and a few ml of redistilled water.

The tissue 5-HT is extracted with 0.4 N perchloric acid in the same way as described for extraction of catechol amines (see above). The time between homogenization and centrifugation should be as short as possible. If this time interval is extended to more than 30 minutes the recovery of 5-HT rapidly decreased, probably due to adsorption of the amine to insoluble tissue residues. After centrifugation of the extract at 0° C, the pH of the supernatant is adjusted to about 6.5 by means of 2 N potassium carbonate solution, and the perchlorate formed spun down at 0° C. The extract is then passed through the column. If not more than 3 ml perchloric acid per gram tissue is used in the extraction procedure, 10—12 ml of the extract can be passed through the column without any breaking through. When the extract has passed, the column is washed with 10 ml of a buffer versene solution (0.02 M phosphate buffer, pH 6.5, containing 0.2 per cent of the disodium salt of ethylene diamine tetraacetic acid) followed by a few ml redistilled water. Elution is performed by means of 3 ml 1.2 N hydrochloric acid. The final determination of 5-HT in the eluate is carried out according to BOGDANSKI et al. (1956). For this purpose 0.8 ml of concentrated hydrochloric acid is added. The fluorescence intensities are read in an Aminco-Bowman spectrophotofluorometer at 316 mμ activating and 565 fluorescent wave-length (uncorrected instrumental values). A UV-filter is applied in the filter holder in front of the photo cell (CARLSSON and MAGNUSSON 1960). This eliminates the light scatter, which otherwise, if high, would interfere with the readings.

Results

As seen in Fig. 1, reserpine (1 mg per kg) caused a depletion of noradrenaline as well as dopamine from the rabbit's brain. The time course of this decrease of the two amines was somewhat different. Dopamine decreased to 50 per cent of its normal value in about 15 min, whereas the corresponding time interval for noradrenaline was about 45 min. The recoveries of the two amines in brain seemed to occur at the same rate which, however, is not seen in the figure. After

two days about 10 per cent of the normal amount was found. On the 9th and 12th about 45 and 65 per cent, respectively, had recovered.

From Fig. 2 it appears that noradrenaline is somewhat more sensitive to low concentrations of reserpine than dopamine.

Reserpine in a dose sufficient to cause a complete disappearance of noradrenaline from the body stores, caused a comparatively slight lowering of the dopamine content in the peripheral organs of the sheep after 13 hrs (Table I). The dopamine in the corpus striatum, however, disappeared completely in that interval of time. Also the amount of 5-HT in peripheral tissues was still remarkably high, whereas the 5-HT in the corpus striatum was reduced to insignificant levels.

Fig. 3 gives the results from the experiments with different drug combinations. It is evident that the dopamine level increased after administration of DOPA to rabbits pretreated with reserpine.[1] This increase was facilitated if the animals had been given monoamine oxidase inhibitors before the DOPA injection. Under the latter conditions a significant increase also of the noradrenaline level was observed.

After treatment with monoamine oxidase inhibitors, the 3-0-methylated metabolite of noradrenaline was observed to occur in brain. It is probable that the corresponding metabolite of dopamine, 3-0-methyldopamine, also was increased to some degree. This metabolite is, however, not included in the present investigation.

Of particular interest is the demonstration of noradrenaline in brain after administration of a monoamine oxidase inhibitor and DOPA. In most of the previous studies aiming to demonstrate that noradrenaline is formed from dopamine, labelled DOPA or dopamin have been used. But, as pointed out by SENOH and WITKOP (1959), the formation of 2,4,5-hydroxyphenyletylamine, with practically the same Rf-value as noradrenaline, may invalidate the results obtained with these methods. In this investigation, however, noradrenaline was determined by a specific fluorimetrical method. The results strongly support the view, that dopamine is the precursor of noradrenaline.

Discussion

Interference with storage mechanism

In order to make the reasoning below clear the metabolism and storage mechanism of the catechol amines has been briefly outlined in Fig. 4. This is of course only a hypothetical picture, based on data now available. The various

[1] In three experiments the penetration of L-DOPA into the rabbit brain was compared to that of D-DOPA. When 100 mg L-DOPA per kg body weight was given i.v. about 10 μg of the amino acid was found per g of brain tissue 15 min after the administration. If the same amount of the D-isomer was given less than 0.5 μg per g of brain could be detected. This observation is in the accordance with the view that the L-amino acids are transferred into the cells by an active mechanism (see LEFEVRE 1955).

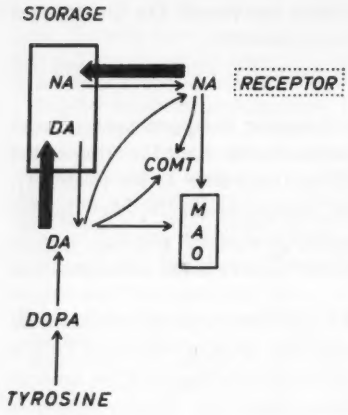

Fig. 4. Hypothetical picture of the metabolism and storage of catechol amines. 1. There seems to be good evidence that the amino acid tyrosine is the precursor of 3,4-dihydroxyphenylalanine (DOPA). 2. DOPA is decarboxylated in the cytoplasm by DOPA decarboxylase. 3. The dopamine (DA) formed is immediately transferred to granules, which probably are of the same type as the adrenaline and noradrenaline (NA) containing particles. In these granules DA can be stored unchanged as in the caudate nucleus. 4. It can, however, also be transformed to NA (*e. g.* in the hypothalamus). 5. The amines are liberated from their storage sites by nerve impulses and then reach their receptors in relatively high concentrations. 6. Outside the granules a continuous inactivation of the catechol amines is going on by means of monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT).

observations concerning the reserpine effect on the catechol amines (and 5-HT) in tissues may be linked together to comprehend only one mode of action. Reserpine may act primarily by blocking the active transport of catechol amines into the granules. The newly formed dopamine is not taken up by the granules. That this is a possible explanation has appeared from preliminary experiments on rabbit adrenal medulla. Differential centrifugation of extracts of this tissue showed, that after injection of DOPA, most of the new-formed dopamine was found in the amine containing granules. If the animals were pretreated with reserpine, however, most dopamine was found in the supernatant and only small amounts in the granular fraction, thus indicating an impaired uptake of the amine formed (BERTLER, ROSENGREN and ROSENGREN 1960, BERTLER, HILLARP and ROSENGREN 1960). These experiments are continued. With the aid of the assumption that reserpine acts by blocking the active uptake of catechol amines by the granules the phenomenon discussed in the following section may be easily explained.

Rate of disappearance of monoamines

The rate of disappearance of catechol amines and 5-HT after reserpine administration seems to be correlated to the turn-over of these amines. Dopamine in the brain appears to have a rapid turn-over as demonstrated by CARLSSON et al. (1959), while adrenaline and noradrenaline in the adrenals have a slow one. In accordance with this view the catechol amines in brain disappeared at a much higher rate than those in the adrenal medulla (cf. CARLSSON et al. 1957 b). This also seems to hold for 5-HT. In the intestine, 5-HT has a half-life of several hours and does not disappear from that organ until 16 hours after the administration of reserpine to the animals. The corresponding figures for brain 5-HT in the

rabbit are both about half an hour (UDENFRIEND 1957, BRODIE 1957). The results in Fig. 1 may thus be interpreted to indicate, that dopamine has an even more rapid turn-over than noradrenaline. From Table I is evident, that the effect of reserpine on dopamine and 5-HT is very similar.

Synthesis of monoamines

The results indicate that reserpine does not affect the formation of catechol amines from DOPA, *i. e.* it does not lessen the DOPA decarboxylase activity *in vivo*. After pretreatment of the animals with reserpine, a DOPA-injection caused an increase of the dopamine level to about the same degree as if DOPA had been given alone, whereas the noradrenaline level still was very low. If the monoamine oxidase had been blocked by nialamid, very high dopamine levels were obtained, and a significant increase of both noradrenaline and normethanephrine as well as 5-HT could be detected. The finding of SHORE et al. (1955) that the excretion in urine of 5-hydroxyindoleacetic acid after a transient increase in reserpinized animals is essentially unchanged, seem to rule out the possibility that reserpine interacts with the synthesis of 5-HT. The slow accumulation of catechol derivatives after the administration of nialamide to rabbits pretreated with reserpine, on the other hand, might suggest that the formation of DOPA from tyrosine were impaired by reserpine. However the formation of 3,4-dihydroxyphenylacetic acid one of the main products of dopamine degradation, seems to proceed at a normal rate in the caudate nucleus of reserpinized rabbits (ROSENGREN 1960). One possibility to explain this apparent discrepancy would be that the synthesis of catechol amines is not impaired by reserpine only but by the combination of reserpine and nialamide.

It is probable, that the ability of the cells to oxidize tyrosine to DOPA and tryptophan to 5-hydroxytryptophan decides which amine will be stored in the different tissues. Some types of suprarenal medullary tumors are able to synthesize DOPA (STUDNITZ and VENDSALU 1960). Patients suffering from carcinoid-tumors, however, do not show an appreciable increase of the principal final metabolite of noradrenaline and adrenaline, *i. e.* 3-methoxy-4-hydroxymandelic acid. Conversely 5-hydroxylindoleacetic acid seems not to be increased in patients suffering from pheochromocytoma (STUDNITZ 1959 and 1960). Aromatic hydroxylation is therefore probably an important step, which deserves further investigation.

Brain monoamines and behaviour

Nialamide seemed to counteract the effect of reserpine on the behaviour of the animals to some degree. Thus animals which had previously been given reserpine in a dose of 1 mg per kg seemed to become quite normal within a few hours after administration of nialamide. From Fig. 3 it is evident that there was a small increase of catechol amines and a larger increase of 5-HT in the brains of those animals. This definitely argues against the hypothesis of BRODIE and co-

workers that the action of reserpine is due to an increase in free 5-HT. It is interesting to note that the rabbits treated with 5 mg reserpine per kg showed much less normalization and also less accumulation of amines. A similar treatment of mice, which had received an even higher dose of reserpine resulted in a slow increase of 5-HT and no alterations in the catechol amine level. These animals did not show any restoration of behaviour (CARLSSON, LINDQVIST and MAGNUSSON 1960).

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From the Finsen Laboratory, Copenhagen, Denmark

Transfer of Radioactive Iodide Between Mother and Foetus in the Rabbit

By

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Abstract

CRONE, M. and G. WAAGØ. *Transfer of radioactive iodide between mother and foetus in the rabbit.* Acta physiol. scand. 1961. *51.* 84—93. — Radioiodide injected subcutaneously into rabbits disappeared more rapidly from the serum of pregnant than non-pregnant animals. To clarify the cause of this difference the ^{131}I uptake in the embryos was measured and higher values were found than corresponded to simple diffusion between mother and foetus. The radioactivity of the foetal gastric juice increased with time. Its ^{131}I concentration was 13 to 109 times higher than in the foetal serum. The ^{131}I concentration in the foetal gastric juice remained high at a time when the ^{131}I concentration had decreased to a low level in the foetal serum. This was considered to indicate that iodide is actively transported through the foetal gastric mucosa and is accumulated in the foetal gastric juice. Radioiodide was found in a higher concentration in the foetal serum and in an even higher concentration in the foetal part of the placenta than in the maternal serum. A steady state of ^{131}I across the placenta was not obtained as the radioactivity of the maternal serum decreased rapidly. A diffusion delay in the foetus was not sufficient to explain the results, which therefore indicated that iodide is actively transported from the mother to the foetus and is concentrated in the foetal part of the placenta. These conclusions were supported by experiments where ^{131}I was administered in such a way that the radioactivity of the maternal serum increased or was constant.

Perchlorate was shown to inhibit the ^{131}I uptake in the foetus by inhibiting the active transport across the placenta and through the foetal gastric mucosa. The iodide concentration mechanism of the foetal part of the placenta was also inhibited.

Radioiodide injected into rabbits, guinea-pigs and rats late in pregnancy is transferred to the embryos and is found in a higher concentration in the foetal than in the maternal serum (JOST, MOREL and MAROIS (1952), HIRVONEN and LYBECK (1956), HALL and MYANT (1956)). LOGOTHETOPOULOS and SCOTT (1956) found that there was no organically bound ^{131}I present in the foetal serum, and concluded that active iodide transport by the placental membrane was responsible for the concentration gradient. NATAF, SFEZ, MICHEL and ROCHE (1956, 1957) have investigated the role of the placenta in the transfer of ^{131}I from the mother to the foetus in rats at the end of pregnancy. They found the ^{131}I concentration temporarily higher in the placenta than in the foetal and the maternal serum. This was taken as evidence that the placenta concentrates radioiodide circulating in the mothers blood and thus ensures transport of iodides to the foetus.

These conclusions could only be drawn provided that a steady state of ^{131}I across the placenta was established. In order to obtain this a rather steady level of radioactivity must be maintained in the maternal circulation. The previous workers administered ^{131}I in different ways (i.v., i.m. and s.c.) but the disappearance curve for ^{131}I from the maternal serum was not shown in any case.

Experiments reported in this study showed that ^{131}I injected subcutaneously into pregnant rabbits disappeared rapidly from the maternal serum and no steady state of ^{131}I could be established across the placenta.

This being the case, the finding of higher radioactivity in the placenta and the foetal serum than in the maternal serum did not prove an active transport of iodide from the mother to the foetus or an iodide concentrating mechanism in the placenta. The ^{131}I might have been transferred to the foetus along a concentration gradient early in the experiment when the ^{131}I concentration was high in the maternal serum, and the findings might be due only to a delay in the passage of ^{131}I from the foetus to the mother.

In the experiments reported in this study the disappearance of ^{131}I from the maternal serum was followed, and it was shown that a diffusion delay of ^{131}I in the foetus was not the only explanation of the higher radioactivity in the foetal samples than in the maternal serum.

The discrepancy between the disappearance of ^{131}I from the serum of pregnant and non-pregnant animals was clarified by estimation of the ^{131}I uptake in the embryos. The uptake of radioiodide in the foetus was found to be very high, and radioiodide was shown to accumulate in the foetal stomach.

Experiments were performed with rabbits at the end of pregnancy. A few animals were used on the 20th day of pregnancy, at a time when the foetal thyroid is supposed to begin to function (JOST, MOREL and MAROIS 1949).

Finally the effect of perchlorate on the transport of ^{131}I from the mother to the foetus was investigated.

Methods

Animals. Young, white female rabbits weighing 3—3.5 kg were used. The number of embryos is large enough and at the end of pregnancy each foetus is so big that a sufficient amount of blood for the various measurements can be obtained.

Experimental procedure. To investigate the transfer of ^{131}I from the mother to the foetus 50 μC ^{131}I (with less than 2 μg NaI) in physiological saline was injected subcutaneously in the axilla. Blood samples were taken regularly from a marginal ear vein to determine the disappearance of ^{131}I from the maternal serum. At different times (from 1.5 hours to 18 hours) after the injection the rabbits were anesthetised with intraperitoneal nembutal sodium (40 mg/kg). If necessary additional ether anesthesia was used. The uterus was opened and each foetus was removed and treated in the following way: Its total radioactivity was measured and the weight recorded. A sample of the foetal blood was taken from the heart. In preliminary experiments radioiodide was found to accumulate in the foetal stomach, and the stomach with its contents was therefore removed. Immediately after the removal of the placenta it was divided into the foetal and the maternal part (HUGGET and HAMMOND 1952) by blunt dissection. Each part was weighed and homogenised in a Waring Blendor with 15 ml distilled water before the radioactivity was measured.

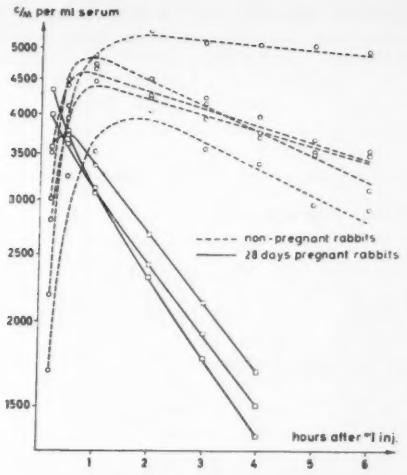
Measurement of radioactivity. The radioactivity of the intact foetus, and in some cases of the stomach and its contents, were measured and related to the injected tracer dose. The radioactivity of the maternal serum, the foetal serum, the foetal gastric juice and homogenates of the foetal and the maternal parts of the placenta were determined after dilution of the samples to equal volumes. A scintillation counter (NaI-Tl-crystal, 40 × 25 mm, background 80 c/m) was used. All samples were counted to a standard deviation of less than 2 %.

Only ^{131}I present as iodide was of interest. To correct for possible organically bound ^{131}I analyses of protein-bound ^{131}I (P.B.^{131}I) and ^{131}I bound to amino acids were carried out. Analyses of P.B.^{131}I were done on samples of maternal serum, pooled foetal serum, foetal gastric juice and placenta homogenates. P.B.^{131}I estimations were done in two ways: a) The proteins of the samples were precipitated with 10 % (w/v) trichloracetic acid. The P.B.^{131}I was calculated as the difference between the radioactivity of the untreatened sample and that of the supernatant solution. b) The samples were dialysed in Visking bags against tap water for 24 hours at room temperature and the radioactivity left in the bags recorded. Analyses of ^{131}I bound to amino acids in placenta and serum were done by paper chromatography, (ROCHE, LISSITZKY and MICHEL 1954) after preparation of the samples according to MYANT (1958). Potassium iodide, monoiodothyrosine, diiodothyrosine, triiodothyronine and thyroxine were used as markers. The radioactivity on the chromatograms was measured by mounting them on a slide which was moved in front of an end-window G-M counter.

Results

Disappearance of ^{131}I from the serum of pregnant and non-pregnant rabbits

Three 28-day pregnant and five non-pregnant rabbits were each given 50 μC ^{131}I subcutaneously. The radioactivity of the maternal serum was measured 10 min, 30 min and 1 hour after the injection and thereafter once an hour. ^{131}I disappeared much faster from the serum of pregnant than from the serum of non-pregnant rabbits (Fig. 1). The biological half-life of ^{131}I in serum was 3 hours in pregnant and 12 hours in non-pregnant animals.



The disappearance of ^{131}I from serum after subcutaneous injection into five non-pregnant and three 28-day pregnant rabbits. The radioactivity is plotted against time.

The transfer of radioactive iodide from the mother to the foetus 28 days after mating

Nine rabbits, 28-day pregnant, with a total number of 63 embryos, were each given 50 μC ^{131}I subcutaneously. From 1.5 hours to 18 hours after the injection the embryos and the placentas were removed. The radioactivity of each foetus (per cent of the injected ^{131}I) was recorded and compared with the per cent of the injected ^{131}I that might be expected in the foetus if ^{131}I were equally distributed between the mother and the foetus, *i. e.* the weight of the foetus in per cent of the mothers weight (the weight of the mother with all the embryos). The mean from each litter is shown in Table I. A significantly higher amount of ^{131}I was found in the foetus than corresponds to simple diffusion. This was studied further by measuring the radioactivity of the maternal serum, the maternal and the foetal part of the placenta, the foetal serum and that of the foetal gastric contents. Table II (rabbits no. 1—9) summarizes the results and shows the maximum radioactivity recorded in the maternal serum. In most experiments this radioactivity of the maternal serum was measured shortly after the ^{131}I injection (Fig. 1).

All the figures in Table II and the following tables represent ^{131}I present as iodide. Less than 2 % was protein-bound in foetal as well as in maternal samples except in the two 18 hours experiments, in which less than 5 % was protein-bound. There was no evidence of ^{131}I bound to amino acids.

In all experiments the radioactivity at the time of removal of the embryos was higher in the foetal than in the maternal serum (Table II, rabbits no. 1—9). Furthermore the radioactivity was higher in the foetal and the maternal parts of the placenta than in the foetal and maternal serum respectively.

Table I. The uptake of ^{131}I in the foetus

Rabbit no.	Litter size	Hours after ^{131}I inj.	Weight of one foetus[1] (g)	^{131}I in one foetus[1] (per cent of the injected ^{131}I)	Weight of one foetus in per cent of the mother's weight[1]	^{131}I in one foetal stomach[1] (per cent of the injected ^{131}I)
28-day pregnant rabbits:						
4	12	1.8	23.1	3.29	0.67	1.62
6	8	4.3	31.4	7.46	1.05	
8	2	18	40.1	7.75	1.23	
9	3	18	29.9	4.90	1.03	
20-day pregnant rabbits:						
13	3	1.7	3.38	0.19	0.10	
14	7	4.4	3.10	0.11	0.08	
28-day pregnant rabbits, after injection of perchlorate:						
15	7	1.5	28.8	0.68	0.82	
16	3	4.3	40	1.10	1.25	
17	9	4.3	29.0	0.82	0.83	0.08

[1] Each figure represents the mean of the litter

Radioiodide was strongly concentrated in the foetal gastric juice. The radioactivity of the foetal serum (excepting the two experiments of 18 hours duration) and of the foetal gastric juice was even higher than the maximum radioactivity of the maternal serum. The radioactivity of the maternal part of the placenta never reached this value.

The transfer of radioactive iodide from the mother to the foetus 20 days after mating

Two rabbits, 20-day pregnant, with a total number of 10 embryos were used. The foetuses and the placentas were removed 1.7 hours and 4.5 hours after the ^{131}I injection. The radioactivity of each foetus was measured and showed slightly higher values than corresponded to equal distribution between the mother and the foetus (Table I). The radioactivity of the different samples are summarized in Table II (rabbits no. 13—14).

1.7 hours after the ^{131}I injection (rabbit no. 13) the radioactivity recorded was the same in the foetal and the maternal serum. In all embryos the radioactivity of the foetal part of the placenta was higher than that of the foetal serum, and that of the maternal part of the placenta was lower than that of the maternal serum. The radioactivity of the foetal part of the placenta and in rabbit no. 14 that of the foetal serum and the foetal gastric juice was

Table II. The transfer of ^{131}I from the mother to the foetus. The table shows the radioactivity (c/m per ml serum or c/m per g tissue) of maternal serum, maternal placenta, foetal placenta, foetal serum and foetal gastric juice

Rabbit no.	Litter size	Hours after ^{131}I inj.	Max. value of mat. serum	Mat. serum at the time of removal of the foetuses[1]	Maternal placenta[1]	Foetal placenta[1]	Foetal serum[1]	Foetal gastric juice[1]
28-day pregnant rabbits:								
1	9	1.5	4,400	2,280	3,380	5,940	6,190	78,700
2	7	1.5	6,230	2,670			6,500	116,200
3	8	1.7	2,700	2,430	2,540	6,160	5,010	102,600
4	12	1.8	4,370	2,250	2,300	9,500	7,100	106,100
5	9	2.0	2,250	2,000	1,930	3,800	3,150	84,700
6	8	4.3	4,020	1,500	2,000	7,010	6,890	746,000
7	4	4.4	3,770	1,500	2,650	6,120	5,380	404,000
8	2	18	3,040	400	490	2,480	2,210	369,000
[2]9	3	18	4,250	320	520	1,900	1,880	209,000
10		1.2	670	670	410	1,360	980	5,630
11		2.6	850	800	670	1,430	1,030	23,200
12		2.5	620	620	480	1,280	1,340	16,000
20-day pregnant rabbits:								
13	3	1,7	5,900	5,150	2,240	6,890	5,150	3,000
14	7	4.4	3,480	2,250	1,900	5,020	4,500	16,500
28-day pregnant rabbits, after injection of perchlorate:								
15	7	1.5	6,000	5,500	3,290	2,390	2,740	1,880
16	3	4.3	7,300	5,750	3,990	2,920	4,890	5,460
17	9	4.3	4,770	4,350	3,290	2,380	3,380	3,370

[1] Each figure represents the mean of the litter

[2] Propylthiouracil is given before the experiment

higher than the maximum radioactivity of the maternal serum. In principle the results were similar to those found 28 days after mating. The ^{131}I uptake by the foetus increased considerably during the last third of pregnancy. Especially the ability of the foetal stomach to concentrate iodide increased.

Accumulation of ^{131}I by the placenta per se

The ability of the placenta to concentrate radioiodide was furthermore studied in experiments in which the transfer of ^{131}I from the placenta to the foetus was prevented by removing the foetus before the ^{131}I injection.

Table III. The concentration of ^{131}I (c/m pr. g) in placentas with the foetuses removed (II) and in placentas with the foetuses left in utero (I)

Rabbit no.	Litter size	Max. value of mat. serum	Foetal serum	Mat. placenta I	Mat. placenta II	Foet. placenta I	Foet. placenta II
18	5	3,300	14,900	4,470	5,510	27,700	52,300
19	4	3,210	8,670	4,700	7,410	12,800	20,600

Two rabbits, 28-day pregnant, were used. The uterus was opened on the opposite side of the placental attachment. One foetus from each of the uterine horns was removed after ligation of the umbilical cord. Great care was taken not to detach the placenta from the uterine wall. The uterus was closed and the rabbit was given 50 μC ^{131}I subcutaneously. The samples were taken 3.8 hours after the injection. Higher radioactivity was found in the placentas without a foetus than in the placentas with the foetus in utero (Table III).

Experiments with ^{131}I injected into the embryos in one of the uterine horns 28 days after mating

Iodide has been shown to be transferred from the foetus to the mother in rabbits, guinea-pigs and rats, (HALL and MYANT (1956), LYBECK and HIRVONEN (1956) and NATAF, SFEZ, MICHEL and ROCHE (1957)). These findings were confirmed by the following experiments. Radioiodide was injected through the uterus and the membranes into the embryos in one uterine horn. Radioiodide was found in the first sample of maternal blood taken 10 min after the injection. The ^{131}I concentration in the maternal serum increased during the first hour after which it remained constant at a level much lower than in the foetal serum (Table IV). This gives a possibility for obtaining a

Table IV. The radioactivity (c/m per ml) of maternal serum and foetal serum after injection of ^{131}I in the foetuses in one uterine horn

Rabbit no.	Hours after ^{131}I inj.	Maternal serum						Serum from injected foetuses[1]	Serum from not injected foetuses[1]
		10 min.	0.5 h	1 h	1.5 h	2 h	2.5 h		
10	1.2	20	300	670				7,350	980
11	2.6	100	500	830	850	830	800	4,160	1,030
12	2.5	50	270	470	600	610	620	7,800	1,340

[1] Mean values

rather steady state of ^{131}I across the placenta of the non-injected foetuses. They were removed 1.2 hours and 2.5 hours after the ^{131}I injection. The ^{131}I concentration was higher in the foetal than in the maternal serum and higher in the foetal part of the placenta and in the foetal gastric juice than in the foetal serum. The ^{131}I concentration in the maternal part of the placenta was lower than in the maternal serum. (Table II, rabbits no. 10—12.)

The effect of perchlorate on the transfer of iodide from the mother to the foetus

Different anions, particularly perchlorate inhibit the active iodide uptake by the thyroid gland (WYNGAARDEN *et al.* 1952) and the active iodide transport through the gastric mucosa (HALMI *et al.* 1956).

Three rabbits, 28-day pregnant, with a total number of 19 foetuses were used to study the effect of perchlorate on the transport of ^{131}I from the mother to the foetus. 200 mg sodium perchlorate were given i.p. 20 min before the ^{131}I injection and additional 100 mg perchlorate 1.3 hours and 2.8 hours later. The rest of the experiments were performed as previously described.

1.5 hours and 4.3 hours after the ^{131}I injection 0.68 % to 1.10 % of the injected ^{131}I was recovered in the foetus (Table I). This was less than should be found in the foetus if ^{131}I was equally distributed between mother and foetus. Comparing these figures with those found in experiments where no perchlorate was given (Table I), it is evident that perchlorate strongly inhibits the ^{131}I uptake in the foetus.

The radioactivity of the different foetal and placental samples was lower than the radioactivity of the maternal serum (Table II, rabbits no. 15—17).

Discussion

The rapid disappearance of ^{131}I from the maternal serum following subcutaneous injection into rabbits late in pregnancy is mainly due to a very high ^{131}I uptake by the embryos. 50 % or more of the radioiodide in the foetus is localized in the gastric juice. The radioactivity of the foetal gastric juice reaches values of more than 100 times that of the foetal serum (Table II, rabbits no. 6, 8, and 9) indicating, that iodide is secreted into the foetal gastric juice.

ELMER (1938) mentioned, that iodide was concentrated in the stomach contents in man. Radioiodide injected intravenously in man (HONOUR, MYANT and ROWLANDS 1952) and different adult animals (LOGOTHETOPOULOS and MYANT 1956) was concentrated in the gastric juice. The last authors also reported that ^{131}I was concentrated in the stomach contents in a 50 days old guinea-pig foetus 1 hour after the ^{131}I injection into the mother.

The experiments presented in this report show, that iodide is secreted increasingly during the last third of the gestation period by the foetal gastric mucosa in rabbit embryos. The radioiodide concentration in the foetal gastric

juice remains high 18 hours after the ^{131}I injection at a time when the ^{131}I concentration is low in the foetal serum (Table II, rabbits no. 8—9). This storage of iodide in the foetal gastric juice does not occur in the adult animal. It can be explained by the absence of transport through the intestinal canal during foetal life and slow reabsorption of iodide through the stomach wall.

Following radioiodide injection into 28-day pregnant rabbits the radioactivity of the foetal serum and the foetal and the maternal part of the placenta is higher than that of the maternal serum at the time of removal of the foetus. During the first 4.5 hours after ^{131}I injection the radioactivity of the foetal serum and of the foetal part of the placenta remains higher than the maximum radioactivity recorded in the maternal serum, but the radioactivity of the maternal part of the placenta does not in any case reach this value (Table II, rabbits no. 1—7). During the same hours the radioactivity of the foetal gastric juice increases significantly. This indicates a net transfer of ^{131}I from the foetal serum to the foetal gastric juice during this period. It is therefore evident that the recording of higher radioactivity in the foetal serum and the foetal part of the placenta than in the maternal serum is not only due to a diffusion delay of ^{131}I in the foetus. It is concluded that iodide is transported from the mother to the foetus against a concentration gradient. Furthermore the radioactivity is higher in the foetal part of the placenta than in the foetal serum indicating, that iodide is accumulated in this part of the placenta. There is no evidence of an accumulation of iodide in the maternal part.

These conclusions are supported by experiments where ^{131}I is injected into the embryos in one uterine horn. In the non-injected foetuses in the other uterine horn higher radioactivity is recorded in the foetal serum and an even higher radioactivity in the foetal part of the placenta than in the maternal serum (Table II, rabbits no. 10—12). This can not be due to a diffusion delay of ^{131}I in the foetus or in the placenta as the radioactivity in the maternal serum is increasing during the first hour and then remains constant during the rest of the experiment. There is no evidence of an accumulation of iodide in the maternal part of the placenta.

The ability of the placenta to accumulate iodide is further studied by removing the foetus before the ^{131}I injection leaving the placenta untouched. Higher radioactivity is found in this placenta than in the placenta with intact foetal circulation (Table III).

It must be mentioned that the measurements of the ^{131}I concentration are carried out on the foetal part of the placenta without correction for its content of maternal or foetal blood, both having a lower ^{131}I concentration than the foetal part of the placenta as a whole. The maternal blood constitutes about 35 % of the foetal part of the placenta at the end of pregnancy (MYANT 1958). The foetal blood volume of the foetal part of the placenta is not known. With these corrections in mind it is seen that the accumulation of iodide in the foetal part of the placenta is more pronounced than our figures indicate.

Perchlorate is shown to inhibit the active transport of iodide across the placenta and across the foetal gastric mucosa, and to inhibit the accumulation of iodide in the foetal part of the placenta (Table II, rabbits no. 15—17). The mode of action of perchlorate is as yet as unknown as the mechanism of active iodide uptake itself.

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Excretion of Catecholamines in Rats Exposed to Cold

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The chemical regulation of heat production which is now well demonstrated in rats has been first postulated to be mediated through the release of adrenaline from the adrenal medulla (MORIN 1948). Recently emphasis has been put on the possible role of noradrenaline as the mediator of the non-shivering heat production (HSIEH and CARLSON 1957). It was then felt important to study the excretion of catecholamines in rats exposed to cold, as a measure of their production.

Rats of the Sprague-Dawley strain weighing 170—180 g were selected. They were kept in wire cages and exposed to a temperature of + 3° C for one month. At all times rats had access to food and water. Controls were maintained at room temperature. Catecholamines in 24 hours urine and in adrenal glands were estimated according to the method of EULER and LISHAJKO (1959).

As seen in the figure rats increase their excretion of noradrenaline very rapidly on exposure to cold. The response is thus nearly maximal already in the first period of 24 hours. The noradrenaline output shows a slow decline with time, but is still four times as high as in the control group after one month in the cold. The adrenaline excretion increases gradually to a maximum in 6 to 8 days and decreases rapidly thereafter.

In the adrenal glands, adrenaline was reduced 25 % after 24 hours in the cold, followed by an increased content which persisted as long as rats were kept in the cold room. The noradrenaline content did not show any significant change.

Adrenalectomized rats excreted as much noradrenaline as intact animals on exposure to cold. However, the adrenaline output in urine, although significantly increased in the cold, was lower than in the control group.

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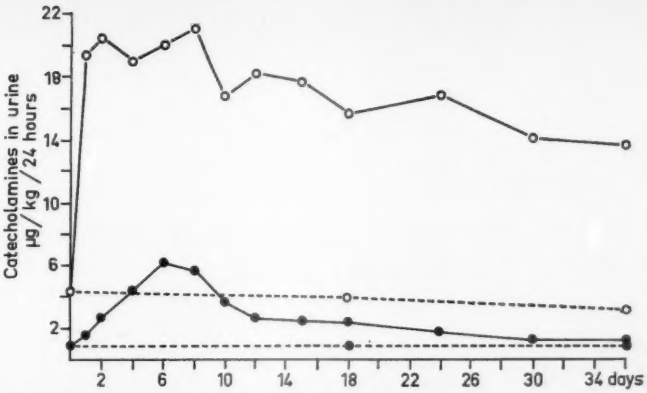

Fig. 1. Excretion of adrenaline (●) and noradrenaline (○) in rats (170—180 g) at +3°C. (———) and +22°C. (— — —). Each point represents the mean of six individual rats.

The large increase in the noradrenaline excretion, and presumably release, on exposure to cold is interesting in view of the strong calorigenic effect of this hormone in cold-acclimated rats as reported recently by HSIEH and CARLSON (1957) and DEPOCAS (1960). The gradual decline in the output may be explained by increasing sensitivity to noradrenaline (cf. DEPOCAS 1960). That noradrenaline is from extra-adrenal origin is shown in adrenalectomized rats. HSIEH, CARLSON and GRAY (1957) have reported that noradrenaline prevents the fall in oxygen consumption caused by hexamethonium in cold-adapted rats. Since there is every reason to assume that noradrenaline derives from the adrenergic nerve endings, it can be inferred that the sympathetic nervous system is involved in the metabolic response to cold-exposure through the release of noradrenaline.

As to the role of adrenaline, it seems to represent a second line of defence which need not be called on for great activity unless the environmental conditions become more severe and/or the other mechanism is exhausted.

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